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THE EFFECTS OF A CONSTANT DOSAGE OF X-RAYS DURING THE PRE-, POST-, AND MID-PROLIFERATIVE PERIODS IN DROSOPHILA MELANOGASTER

J. D. COMBS AND H. L. GRAVETT

University of Georgia School of Medicine, Augusta, Georgia
and
University of Illinois, Urbana, Illinois

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INTRODUCTION

THE demand for knowledge of the effects of X-ray irradiation upon somatic tissues has become increasingly important from the standpoint of its therapeutic value. To this end, the efforts of practically all branches of biological science have been summoned. The accumulation of considerable X-ray data by geneticists and embryologists has aided materially in a better understanding of cellular physiology. It has been pointed out by BARDEEN (1910) that the effects of X-rays upon cells is especially associated with changes in the nuclear material and that these changes occur chiefly during the pre-mitotic stages. MULLER (1928) and PATTERSON (1929) have given cytological evidence of the destructive effects of X-rays upon the nuclear structure. BARDEEN (1910) has given a physiological classification of the effects of X-rays in which he distinguishes between sensory-motor, morphogenic, and metabolic effects. REGAUD and DUBREUIL (1908), on the other hand, based their classification of X-ray effects upon morphological grounds by showing that X-ray effects may be cyto-hereditary, in which the effects are manifested in the daughter cells or cells of a more remote descent, and direct effects or cellular destruction.

In an attempt to analyze certain phases of development of an organ the authors have carried out experiments which show that during the pre-proliferative and proliferative periods 1325 X-ray units are effective in producing marked changes in the character of an organ while during the post-proliferative period there is little or no effect of the same dosage of X-rays upon the structure under observation.

MATERIALS AND METHODS

Because of the ease with which Drosophila may be reared, the availability of highly inbred stock, its brief period of development, the lack of maternal influence through fetal membranes, and the more accurate knowledge of the developmental period of the wing, this animal was chosen for the experimental material. One of the authors (COMBS 1937b) has

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determined the developmental period of the wing of Drosophila under rigidly controlled conditions. The method employed was physiological, rather than from stained histological sections. It is believed that this method is more accurate inasmuch as it eliminates the possibility of overlooking the earliest primordium and the close of the proliferation, as is likely to occur in sectioned material.

The usual methods of Drosophila workers were employed in rearing the flies. A description of the preparation of the cultures, the temperature controls, and the determination of the developmental period have been given elsewhere (COMBS 1937a). The temperature was maintained by automatic electric incubators housed in the temperature rooms of the University of Illinois. The recording apparatus showed that the temperature was maintained at $27 \pm .3$°C throughout the experiment except during the time when the embryos were being X-rayed. At this time the developing flies were carried to the X-ray rooms and were at a slightly higher temperature for a period of approximately 45 minutes, after which they were returned to the incubator where they were allowed to complete their development. The embryos were produced by parents which had been inbred for 357 generations of brother and sister matings and had been kept at 27°C throughout this long period of inbreeding.

The X-ray was equipped with a tungsten target and an aluminum filter 1 mm in thickness. The dosage in each group of different developmental stages was 1325 r units as calibrated by use of a Victoreen dosimeter. The embryos were developed in cotton plugged vials in which 1½ inches of banana-agar-yeast served as food medium. It was realized that these containers induced some error in the dosage of individuals deep in the food as compared with those which had crawled upon the sides of the vials. However, the number of individuals used made other techniques impractical. The target was directed so that the rays passed through the cotton plugs and the dosage was calculated upon the basis of the average distance of the larvae from the target at the onset of irradiation.

It has been shown (COMBS 1937b) that the active period of the proliferation of cells in the production of the wing probably begins at the 72nd hour and ends at the 144th hour if the embryos are kept at a constant temperature of 27°C during the entire development. The present experiments were planned so that four groups of embryos were X-rayed—two before the active stage of proliferation of the wing cells, one during the active proliferation, and one after the proliferating stage had ended. A fifth group was not X-rayed and it served as a control group.

After the imagoes appeared they were allowed to remain at room temperature for 24 hours in new vials of food which had been prepared in advance and had dried sufficiently to keep the wings from adhering to the

vials and to keep the flies from becoming entangled in the food. They were then etherized lightly and examined under a dissecting microscope. Records were made of the imagoes which showed wing defects. The total number of flies which emerged, the number of pupae which failed to open and the sexes of all individuals were recorded. Observations were made on the individuals which emerged during the first 24 hours and those which emerged during the second 24 hours, except in the control group where both of these periods were combined into a single one. All flies were preserved in 95 percent alcohol. No absolute measurements were made on the wings and records were only made of the defective wings which were immediately visible under the dissecting microscope. The defects most frequently encountered were shrivelled or somewhat crumpled wings. Such a condition was often distributed over the entire wing but occasionally it was localized in a small region either on the margin or within the wing. Another type of defect found in noticeable numbers was characterized by a curling of the wing. Sometimes the curling was dorsalward, similar to the mutant ski, and in practically as many cases they were curved ventrally. Since the wing is composed of only two layers of cells it is easy to see how injuries to the dorsal layer of cells only might cause a dorsal curling or, in case of a ventral layer injury, a ventral curling. A few showed distinct abnormalities in the manner in which the wings were carried. This was usually accompanied by visible crumpling of the region near the attachment of the wing to the body. Bilateral defects in the wings were common but not always of the same nature or degree. In one case, an entire wing was absent. This was associated with a thoracic deficiency. A total of 1793 individuals which emerged in the experimental groups were observed. This was an average of 448 individuals per group. In the control group there were 256 individuals. In the experimental groups the number that emerged fell considerably short of the number of embryos which were treated since many probably died before pupation and from the pupae counts it was found that only abut half of the individuals emerged during the 48 hours in which the imagoes were collected. After that period a few more emerged but they were not examined since the culture conditions might render the data unreliable. Many of those individuals which emerged after the 48 hour period were apparently so badly injured that they became stuck in the food and this had a pronounced effect upon the unfolding of the wings.

Under ordinary circumstances, most of the pupa cases should have been vacated within the 48 hour period since all of the eggs were collected in a period of two hours. However, in the X-rayed groups about half of the puparia were still occupied after the 48 hour period. On the basis of data to be presented it seems that many of those that did not emerge had defects too serious to survive. Large numbers succeeded in partially freeing

themselves from the puparia but were unable to carry the process to completion.

EXPERIMENTAL DATA

MAVOR (1927) has called attention to the sexually dimorphic effects of X-rays upon Drosophila. He has shown that the males are considerably more susceptible to lethal effects of irradiation than females. Our table 1 leads to a similar conclusion. It has been found (unpublished data) that the stock (Bar, forked) used in these experiments does not give the expected sex ratio of 1:1. Since the mutant characters carried are sex-linked they may be responsible for the variation from the expected ratio. Of those individuals not X-rayed the ratio of males to females was 43:57. In the X-rayed groups, for the entire period of emergence studied, the ratios ranged from 26 males:74 females to 33 males:67 females. These facts appear to confirm the conclusion of MAVOR concerning the selective lethal effect of X-rays upon the two sexes. This has been interpreted, in part at least, to be the result of induced lethal recessives in the X chromosome which are overshadowed by the viable alleles in the homologous chromosome in the females. No experiments were made to test this conclusion. Table 1 shows also that with the exception of the 70 hour group the ratio of the second 24 hours of emergence comes nearer to the expected ratio than the first 24 hour period.

TABLE 1

Sex ratio.

HOURS OF DEVELOPMENT WHEN X-RAYED	PERIOD EMERGED					
	1ST 24 HOURS		2ND 24 HOURS		TOTAL 48 HOURS	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
50	29.2	70.8	43.1	56.9	33.2	66.8
70	42.1	57.9	37.4	62.6	26.1	73.9
97	25.0	75.0	50.7	49.3	32.8	67.2
167	25.2	74.8	36.9	63.1	30.1	69.9
NOT X-RAYED					42.6	57.4

Table 2 shows the percentage of individuals which emerged that showed wing defects. In the X-rayed groups, except for the 167th hour, about three times as many defective flies emerged during the second 24 hours as during the first 24 hours. It is also obvious that the total number of defects in the group X-rayed at the 167th hour is not different from those which were not X-rayed. The total number of defects among those individuals X-rayed at the 50th, 70th, and 97th hours of development is about three times as great as the group which was X-rayed at the 167th hour of de-

velopment and also as the group which was not X-rayed. This would seem to indicate that X-ray injuries prolong the developmental period.

TABLE 2

Percentage of flies emerging with unilateral or bilateral defects.

HOURS OF DEVELOPMENT WHEN X-RAYED	PERIOD EMERGED		
	1ST 24 HOURS	2ND 24 HOURS	TOTAL 48 HOURS
50	4.2	11.9	6.3
70	0.9	13.5	7.4
97	2.8	12.3	6.7
167	2.4	3.7	2.8
NOT X-RAYED			2.7

In table 3 it is found, in general, that the percentage of male defectives falls considerably short of the percentage of female defectives. It seems that this is probably due to the greater susceptibility of the males to the lethal effects of X-rays. It is highly suggestive that many of the males with defective wings were probably not observed. While some inconsistencies are seen in this table one would be inclined to conclude that for the 48 hour period of emergence there is a similar sex ratio of defectives in the various X-rayed groups as well as in the control group, except perhaps in the 97 hour group. Whether this group is significantly different from the others is questionable because of the relatively small number of individuals observed.

TABLE 3

Sex ratio of defective individuals.

HOURS OF DEVELOPMENT WHEN X-RAYED	PERIOD EMERGED					
	1ST 24 HOURS		2ND 24 HOURS		TOTAL 48 HOURS	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
50	26.7	73.3	11.1	88.9	18.2	81.8
70	00.0	100.0	20.0	80.0	18.7	81.3
97	25.0	75.0	33.3	66.7	28.6	71.4
167	38.5	61.5	8.0	92.0	18.4	81.6
NOT X-RAYED					14.3	85.7

Each wing may be considered as a separate developmental unit and the cases in which the wings of both sides are defective may be weighted equally with those of unilateral defects or bilaterally uninjured wings. Table 4 gives the data derived from such consideration. Here again it is

found that the defective winged individuals are greatly delayed in emerging since the 50, 70, and 97 hour groups have several times as many defects in the individuals which emerged in the second 24 hour period as emerged in the first 24 hours. When both periods are combined it is found that the 50, 70 and 97 hour groups have considerably more defective wings than the 167 group; and that the 167th hour and the untreated groups were similar in having very few defects present. This seems to be the most significant result of the entire investigation.

TABLE 4

The percentage of defective wings observed, considering each wing as a separate unit.

HOURS OF DEVELOPMENT WHEN X-RAYED	PERIOD EMERGED		
	1ST 24 HOURS	2ND 24 HOURS	TOTAL 48 HOURS
50	3.2	9.8	5.4
70	0.7	11.2	5.9
97	1.8	11.0	5.6
167	1.8	2.8	1.6
NOT X-RAYED			1.8

DISCUSSION

In a preliminary report, COMBS (1937c) has pointed out that X-rays have a destructive effect upon the development of the wings of Drosophila. The same author (1937b) has shown that the proliferative period of the wings of flies reared at 27°C extends from the 72nd to the 144th hour of development. Data have been presented which indicate that X-rays are effective previous to this period of proliferation. The question of the nature of the pre-proliferative period effect is as yet a speculative one. It is possible that the genetic factors concerned with wing production are affected previous to their active stage in differentiation and that they are unable to function properly in this process when such a time has arrived. On the other hand, it is possible that such an effect is merely an interference in the active stage of the genes in which the organ-forming substance is not produced in its normal manner. It does seem evident that after the proliferative period (after the 144th hour) X-rays, of the dosage used in these experiments, are not significantly effective in producing wing defects.

MAVOR (1927) has pointed out, on the basis of the lethal effects of X-rays, that a period begins at the time of pupation when the X-ray dosage must be markedly increased to produce the same results as at earlier stages of development. At 27°C it has been found that pupation in this stock occurs (COMBS 1937b) at about the 99th hour of development.

At this period the differentiation of tissues in Drosophila is considerably advanced. It has been suggested by BARDEEN (1910) that the most actively proliferating tissues are those which are subject to the greatest effects of X-rays. If most of the vital organs of Drosophila are well differentiated at this time it seems reasonable to assume that X-rays would be less effective, except in increased dosage in which the formed tissues would be destroyed, in producing detrimental morphological changes. However, the present data seem to indicate that pre-proliferative effects, particularly when the beginning of proliferation is relatively late for the given organ, is also an effective period for X-rays and it seems probable that these effects are caused by nuclear derangements which become manifested in the structures concerned at a later period as a result of the interference with the normal process of differentiation.

The senior author wishes to express his sincere appreciation to the Zoology Department of the University of Illinois for their kindness in placing at his disposal the Zoological Laboratory and its equipment. Both of the authors are indebted to the Chemistry Department of the University of Illinois for the use of their X-ray equipment. Especially the authors wish to thank Dr. J. N. MRGUDICH for his technical assistance in the use of the X-ray machine.

SUMMARY

1325 r units of X-rays were found to produce a significant number of defects in the wings of Drosophila during the pre- and mid-proliferative periods.

The same dosage of X-rays was not effective in producing wing defects during the post-proliferative period of wing development.

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THE EXTRA CHROMOSOME OF OENOTHERA LAMARCKIANA LATA

D. G. CATCHESIDE

International Fellow of the Rockefeller Foundation
California Institute of Technology, Pasadena, California

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INTRODUCTION

OENOTHERA LAMARCKIANA LATA is one of the original "mutations" obtained by DE VRIES and found by LUTZ (1909, 1912) and GATES (1912) to have an extra chromosome. HÅKANSSON (1930) showed that the extra chromosome was identical with one of the chromosomes in the ring of twelve, but he had no further evidence to show which of these chromosomes was duplicated. The ring of twelve and extra chromosome configuration was most frequently represented at diakinesis and metaphase I of meiosis by a ring of twelve chromosomes and a univalent, or a chain of thirteen chromosomes or a chain of eleven chromosomes and a ring pair. In the last case, the two similar chromosomes from the ring of twelve would always segregate to opposite poles at anaphase I; in the other cases the chances are equal that they would pass to opposite poles or to the same pole. It follows that the extra chromosome more often than not must disjoin from that complex containing its structurally identical homologue. As pointed out by EMERSON (1936) and CATCHESIDE (1936), the genetical evidence shows that the additional chromosome of *lata* comes from the *gaudens* complex. For, *gaudens* gametes are produced more frequently relative to the *velans* gametes, than in the corresponding diploid, and the *velans*+*lata* gametes are much commoner than the *gaudens*+*lata* gametes.

It is the purpose of this paper to present evidence leading to the identification of the extra chromosome in terms of the interchanges defined by EMERSON and STURTEVANT (1931). The homologies of the pairing ends of the extra chromosome have been determined by analyzing the chromosome configurations at meiosis (at diakinesis and metaphase I) in pollen mother cells of trisomic individuals compounded of various known, or partially known, interchanges together with the extra (*lata*) chromosome. The constitutions of the different complexes used are given at the appropriate place in the text. Several are based on data that are partly unpublished. In their identification, one alteration of the definitions of chromosome ends proposed by EMERSON and STURTEVANT has been made. Here the separation of 5, 6, 7, 8, 9, 10, 13 and 14 is made to depend on *albicans* instead of *jugens*, as much more data involving the former complex are now available. *Albicans* is therefore 1·4, 7·14, 8·9, with the remaining four chromosomes only partially identified.

I am grateful to Prof. T. H. MORGAN for providing me with facilities for this investigation and to Prof. A. H. STURTEVANT and S. H. EMERSON for discussion and criticism of the problem.

GENETIC CONSTITUTION OF OENOTHERA LAMARCKIANA LATA

The *lata* strain used in these experiments arose as a primary non-disjunction in a family of *r-Lamarckiana* plants descended from seed received from Prof. HUGO DE VRIES. In outcrosses in which *lata* was female parent, *velutina* and *laeta* twins were in nearly equal proportions, respectively 37 percent (125 plants) and 31 percent (107 plants) of the total progenies reaching maturity. Amongst the trisomics in the outcross progenies, *velutina-lata* were much the commonest, making 25.4 percent (88 plants) of the total progeny; *laeta-lata* were rare, amounting to merely 0.6 percent (2 plants). In addition, there were some 6 percent (20 plants) some of which were new trisomics, others new interchanges, others triploid and still others of an unidentified nature; some of these have been analyzed cytologically. Some 147 seedlings, not included in the figures above, died before they could be classified; some were genetic chlorophyll deficients (chiefly *velans* combinations), but the others were the victims of an attempt to induce earlier summer flowering by means of January sowings. Many seedlings were too weak to make progress in the brief light hours, often of low intensity, characteristic of London at that period of the year.

The strain of *Lamarckiana*, from which the trisomic arose, usually has relatively few *gaudens* megaspores functioning in comparison with the number of *velans* megaspores. One series of nine different pollinations in which both types should have survived successfully, gave 209 *velutinae* (86 percent) and 34 *laetae* (14 percent); this series, however, showed the usual wide fluctuations characteristic of this particular megaspore competition.

In comparison, the progenies of the *lata* plants show a proportionately greater frequency of *gaudens* megaspores than do those of the corresponding diploids. Also, eight chromosome complexes involving *velans* are much commoner (44 times more frequent) than those involving *gaudens*. Both these properties indicate, as shown by EMERSON (1936), that the extra chromosome is one from the *gaudens* complex. Moreover, this extra chromosome must be from the ring of twelve since, in an individual trisomic for the pairing chromosome (1·2), the extra chromosome would not tend to go more often with *velans* than with *gaudens*.

The characters of the *lata* trisomic must be due to the change of balance consequent upon the introduction of the extra chromosome. In final analysis, no doubt, this can be expressed largely in terms of the genes

carried by the extra chromosome concerned. For simplicity, we may anticipate the cytological findings that the extra chromosome in *lata* is 5·6. In point of fact, no genes are known to lie in *gaudens* 5·6. The two major characters of *lata*, namely its male sterility and its broad, blunt leaves and associated characters, may be supposed to be dependent on recessive genes located in *gaudens* 5·6. Perhaps their normal alleles for male fertility and pointed leaves are located in *velans* 5 and 6. We should note that all the *lata* individuals in the outcrosses I have studied were male fertile. This may indicate that the putative male sterility gene is confined to *gaudens* amongst the complexes studied. Pollen fertile races of *lata* described in the literature are without significance in this connection as they resulted from the crossing of *lata* with other unanalyzed, *lata*-like forms. The other supposed gene giving the blunt, broadly pointed leaves and stout blunt buds would seem to bear some relation to the recessive *sp* of *rubens*, also located in 5·6 (RENNER, 1933). But the effects of the *gaudens* gene, if it be such, are much less extreme than are those of *rubens sp*. Thus *velans·rubens lata* had extremely blunt leaves and buds. In *velans·flavens lata*, in which *flavens* 5·6 carries *Sp* (RENNER 1933), the leaves and buds were broader and stouter than in *velans·flavens* but scarcely blunt. The *ʰHookeri* and *ʰpurpurata* combinations with *velans-lata* had rather blunter leaves and buds. The *ʰblandina* and *rubricalyx α* combinations with *velans lata* were almost as blunt-leaved and blunt-budded as *Lamarckiana lata*. Further, *ʰblandina·gaudens lata* was more extreme in blunting than *Lamarckiana lata*, but less so than *velans·rubens lata*. The situation rather suggests that different complexes have different multiple alleles corresponding to *sp* of *rubens* and *Sp* of *flavens*, which may be considered as the two extremes, and that a simplex dominant may be overruled by two doses of relatively recessive alleles. However, the other differences between complexes render such speculations dubious in the absence of corroborative evidence. In particular, I know of no recorded cases of segregation of male steriles or of blunt-leaved individuals in *laeta* combinations, in which 5·6 is a pair. A specific test of this point must be made in such a way that we may be certain that *gaudens* 5·6 is segregating out in homozygous condition.

SUMMARY OF PROGENIES

1. *r-Lamarckiana* selfed. Seed from Prof. H. DE VRIES, 1931. First generation 1931, 3. Second generation, 8 *r-Lamarckiana* and 1 *lata*, 1932, 19.

2. *lata* by *r-Lamarckiana*. 5 *lata* and 13 *r-Lamarckiana*, 1934, 1; 2 *lata* and 10 *r-Lamarckiana*, 1934, 245; 3 *r-Lamarckiana*, 1934, 246.

3. *lata* by *r-Lamarckiana*. 1 *pallescens* and 5 *r-Lamarckiana*, 1935, 130.

4. *lata* by *r-Lamarckiana*. 26 seeds gave 1 *lata* and 1 *r-Lamarckiana*, 1936, 119; 50 seeds gave 3 *lata* and 2 *r-Lamarckiana*, 1936, 120; 74 seeds gave 3 *lata*, 4 *r-Lamarckiana*, 1 *albida* and 1 small rosette with growing point gone blind (neither of latter flowered), 1936, 121; 58 seeds gave 1 *lata*, 8 *r-Lamarckiana*, 1 doubtful *albida*, 1 small rosette with long petioles and nearly round leaf blades and 2 unidentified rosettes (none of the last four flowered), 1936, 122; 53 seeds gave 7 *lata*, 16 *r-Lamarckiana* and 1 *albida* (not flowered), 1936, 124; 83 seeds gave 1 *lata*, 12 *r-Lamarckiana* and 1 *rubrinervis*, 1936, 125; 77 seeds gave 5 *lata*, 10 *r-Lamarckiana*, 1 *rubrinervis* and 1 rosette with longly petiolate leaves (not flowered), 1936, 126; 61 seeds gave 5 *lata*, 6 *r-Lamarckiana*, 1 *rubrinervis* and 1 *albida* (not flowered), 1936, 127.

5. *lata* by *suaveolens*. 38 seeds gave 4 *velans·flavens* and 8 pale seedlings died, 1936, 96; 43 seeds gave 2 *velans·flavens*, 1 *gaudens·flavens* and 6 pale seedlings died, 1936, 97; 35 seeds gave 2 *velans·flavens*, 1 *velans·flavens lata* and 14 pale seedlings died, 1936, 98.

6. *lata* by *blandina*. 90 seeds gave 28 *velans·ʰblandina*, 9 *gaudens·ʰblandina*, 6 *velans·ʰblandina lata* and 15 pale seedlings died, 1936, 99; 56 seeds gave 19 *velans·ʰblandina*, 2 *gaudens·ʰblandina*, 13 *velans·ʰblandina lata*, 2 pale yellow green rosettes (not flowered) and 2 pale seedlings died, 1936, 100; 64 seeds gave 8 *velans·ʰblandina*, 1 *gaudens·ʰblandina*, 17 *velans·ʰblandina lata*, 1 *gaudens·ʰblandina w* (trisomic), 1 *velans·ʰblandina y* (trisomic), 1 diploid dwarf, 1 small pale green rosette (not flowered) and 1 pale seedling died, 1936, 101; 80 seeds gave 9 *velans·ʰblandina*, 15 *gaudens·ʰblandina*, 16 *velans·ʰblandina lata*, 3 rosettes of two unknown types (not flowered) and 12 pale seedlings died, 1936, 102; 90 seeds gave 25 *velans·ʰblandina*, 14 *gaudens·ʰblandina*, 10 *velans·ʰblandina lata*, 2 *gaudens·ʰblandina lata*, 1 *velans·ʰblandina y* (trisomic), 1 *velans·ʰblandina z* (trisomic), 1 pale green rosette (not flowered) and 14 pale seedlings died, 1936, 103.

7. *lata* by *blandina B*. 50 seeds gave 7 *velans·ʰblandina B*, 11 *gaudens·ʰblandina B*, 1 *velans·ʰblandina B lata* and 12 pale seedlings died, 1936, 104; 50 seeds gave 9 *velans·ʰblandina B*, 5 *gaudens·ʰblandina B*, 6 *velans·ʰblandina B lata*, 2 plants of unknown type and 4 pale seedlings died, 1936, 105.

8. *lata* by *purpurata*. 54 seeds gave 6 *velans·ʰpurpurata*, 9 *gaudens*, *·ʰpurpurata*, 9 *velans·ʰpurpurata lata*, 1 pale rosette (not flowered) and 14 pale seedlings died, 1936, 106; 61 seeds gave 3 *velans·ʰpurpurata*, 11 *gaudens·ʰpurpurata*, 1 *velans·ʰpurpurata lata*, 2 rosettes (not flowered) probably trisomics and 23 pale seedlings died, 1936, 107.

9. *lata* by *Hookeri*. 40 seeds gave 6 *gaudens·ʰHookeri*, 1 *neovelans·ʰHookeri*, 3 *velans·ʰHookeri lata* and 3 pale seedlings died, 1936, 108;

47 seeds gave 10 *gaudens·ʰHookeri*, 1 *velans·ʰHookeri lata*, 1 pale green rosette (not flowered) and 18 pale seedlings died, 1936, 109.

10. *lata* by *rubricalyx α*. 36 seeds gave 2 *velans·rubricalyx α*, 7 *gaudens·rubricalyx α*, 4 *velans·rubricalyx α lata* and 1 rosette died, 1936, 110; 38 seeds gave 1 *velans·rubricalyx α*, 6 *gaudens·rubricalyx α* and 1 pale seedling died, 1936, 111.

11. *lata* by *muricata*. 30 seeds gave 1 probable *velans·curvans* and 3 *velans·curvans lata* (none flowered), 11 seedlings that lacked chlorophyll entirely and died early and 2 seedlings that had some chlorophyll but died later, 1936, 112.

12. *lata* by *biennis*. 10 seeds gave 2 *velans·rubens lata*, 1936, 113; 22 seeds gave 1 *velans·rubens*, 4 *velans·rubens lata* and 3 seedlings died, 1936, 114.

13. *lata* by *eriensis*. 43 seeds gave 10 seedlings that lacked chlorophyll and died and 1 green tetrasomic triploid of constitution *velans·gaudens·undulans lata*, 1936, 115; 28 seeds gave 8 chlorophyll-less seedlings that died, 1936, 116.

14. *lata* by *angustissima*. 17 seeds gave 5 white seedlings that died and 1 chimera, with one white cotyledon and the other nearly all green, that grew for a time and later died, 1936, 117; 14 seeds gave 4 white seedlings that soon died, 1 seedling with some chlorophyll that survived for a time and 1 green tetrasomic triploid of the constitution *velans·gaudens·divergens lata*, 1936, 118.

CYTOLOGY OF THE O. LAMARCKIANA LATA PROGENIES

The cytological observations are based exclusively on permanent smears of pollen mother cells fixed in Belling's modification of Navashin's fluid and stained by the gentian violet-iodine-chromic acid technique. Only diakinesis and metaphase I stages were studied.

The constitutions of some of the complexes used (*gaudens*, *ʰblandina*, *ʰpurpurata* and *rubricalyx α*) are based largely upon unpublished data of Prof. S. H. EMERSON, Prof. R. E. CLELAND and myself. They are to some extent tentative. Chromosomes, to the identification of which any doubt attaches, are enclosed in brackets. The formulae of the remainder are taken from EMERSON and STURTEVANT (1931).

ʰHookeri	1·2	3·4	5·6	7·8	9·10	11·12	13·14
flavens	1·4	2·3	5·6	7·8	9·10	11·12	13·14
velans	1·2	3·4	5·8	6·7	9·10	11·12	13·14
gaudens	1·2	5·6	(3·13	4·10	7·12	8·14	9·11)
rubens	1·2	5·6					
ʰblandina	1·2	3·4	5·6	7·10	(8·11	9·12	13·14)
ʰblandina B	1·4	2·3			rest like *ʰblandina*.		

ʰpurpurata	1·2	3·4	5·6	11·12	(7·10	8·9	13·14)
rubricalyx α	1·2	3·4	5·6	9·10	(7·12	8·11	13·14)

Diploids

The configurations found in the diploid *velutina* and *laeta* combinations in a number of the progenies of *O. Lamarckiana lata* pollinated by various standard strains are as follows:

velans·flavens 4, 4, 2, 2, 2
velans·ʰblandina 8, 2, 2, 2
velans·ʰblandina B 8, 4, 2
velans·ʰpurpurata 6, 2, 2, 2, 2
velans·Hookeri 4, 2, 2, 2, 2, 2
velans·rubricalyx α 6, 2, 2, 2, 2
velans·rubens 12, 2
velans·gaudens 12, 2
gaudens·flavens 12, 2
gaudens·ʰblandina 10, 2, 2
gaudens·ʰblandina B 12, 2
gaudens·ʰpurpurata 10, 2, 2
gaudens·ʰHookeri 10, 2, 2

All of the configurations agree with those previously known for the same combinations and therefore suggest that the *velans* and *gaudens* gametes of *O. Lamarckiana lata* have their standard interchange formulae.

In one pollen mother cell of *gaudens·flavens* a case of a figure of eight was observed (fig. 4). It was a disjunctional example of the type resulting from crossing over between corresponding segments of opposite complexes. The new interchange complexes would have been inviable, since each would have had one segment duplicated and a second one deficient. Viable gametes could have resulted only if there had been an associated non-disjunction with the formation of an eight-chromosome gamete.

One exceptional *velutina*-like individual (recorded above as *neovelans ·ʰHookeri*) in a family from *lata*×*Hookeri* was found to have seven pairs of chromosomes at meiosis. *Neovelans* therefore had 5·6, 7·8 in place of 5·8, 6·7 (usual for *velans*) and is an example of a new interchange. It must have arisen from crossing over between homologous segments in non-corresponding arms in the 8·5, 5·6, 6·7 region of the ring of twelve in *O. Lamarckiana*. The required structure of this region would be A·8, 8·x5, 5·6, 6·7, 7x·B or A·x8, 8·5, 5·6, 6x·7, 7·B, where x and x· are the homologous interstitial segments and A and B are unspecified pairing segments of *gaudens* chromosomes. It is interesting to note that the "*deserens*" segregates from a strain of "*rubrinervis*," obtained from Prof. DE VRIES in 1930, also gave seven pairs with *ʰHookeri* and a ring of four with *velans*. *Rubrinervis* and *deserens* are not single entities; structurally different ones look alike and are usually referred to as being the same.

Trisomics

(a) *lata* trisomics

(i) *velans·gaudens lata*. Nine *Lamarckiana lata* individuals have been

examined cytologically. The critical configurations seen were of the following kinds: (a) a chain of thirteen chromosomes and a ring pair (b) a rod chromosome attached by one end to a ring of twelve chromosomes and a ring pair and (c) a chain of eleven chromosomes and two ring pairs. The presence of a closed ring of twelve chromosomes in many pollen mother cells proves that complete *velans* and *gaudens* sets were present in the trisomics. The occurrence of a chain of thirteen chromosomes, as well as cases of attachment of the extra chromosome to a ring of twelve chromosomes, shows that the *lata* chromosome has a pairing end, or ends, in common with the ring of twelve chromosomes. It is independent of the free

FIGURES 1–4. "Figures-of-eight" at metaphase I in pollen mother cells of *Oenothera*. Figures 1–3 *Lamarckiana lata;* figure 4 *gaudens · flavens*. ×4000.

pair (1·2). Finally, cases of two ring pairs, one of which must represent the pair in diploid *Lamarckiana*, show that the extra chromosome is identical with one of the ring of twelve chromosomes. The cytology of *lata* is therefore in complete agreement with the genetical data; it is a typical dimorphic trisomic. The gametes produced by *lata*, apart from new interchanges and new non-disjunctional combinations, will be *velans*, *gaudens*, *velans lata* and *gaudens lata*.

The usual chromosome configurations, in which the chromosomes are associated by completely terminalized chiasmata have been sufficiently illustrated by HÅKANSSON (1930). Certain special types, namely figure-of-eight configurations involving chiasmata between homologous interstitial regions in otherwise non-homologous chromosomes, are of importance since they are the basis of new interchanges. Figures 1–3 illustrate three

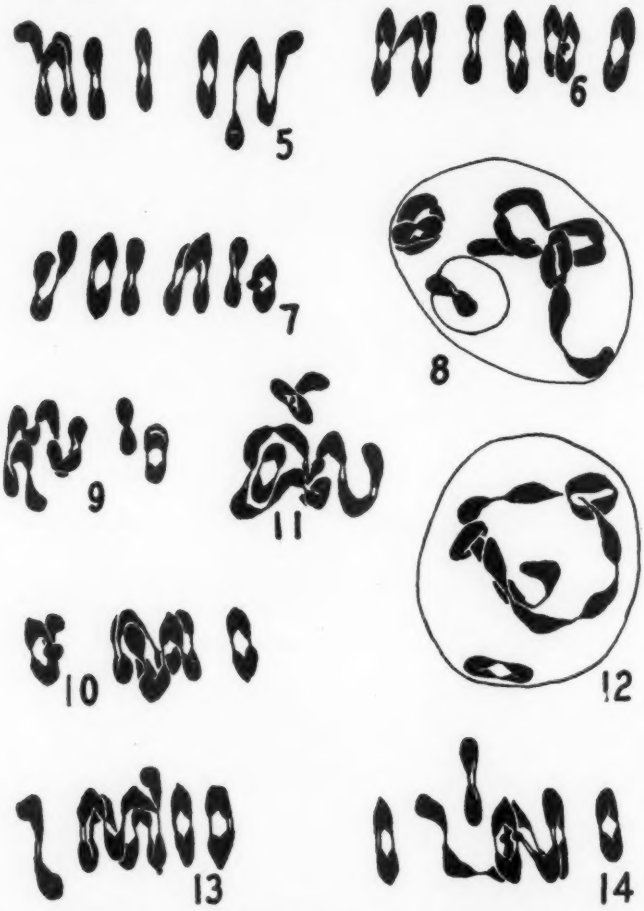
examples, two at metaphase I and one (fig. 3) at anaphase I; the last shows the exceptional amount of lagging induced between chromosomes associated by an interstitial chiasma. The genetical implications of these configurations have been fully discussed by DARLINGTON (1932) and EMERSON (1936). In trisomics, the presence of the extra chromosome inhibits any attempt to determine the relative positions of the homologous interstitial segments. These configurations are more frequent in *lata* (15 p.m.c. out of 92 analyzed) than in diploid *Lamarckiana* (about 1 p.m.c. per 200). This is reflected in a greatly increased frequency of new interchanges, such as those in half-mutants, amongst the progenies of *lata*. Thus, the progenies recorded above show, from *lata*×*Lamarckiana*, 90 *Lamarckiana*, 33 *lata*, 3 *rubrinervis*, 4 *albida* and six of other types. The frequency of *rubrinervis* segregates is 2.2 percent, compared with about 0.1 percent in *Lamarckiana* progenies. Other *Lamarckiana* trisomics show a similar behavior but it is not known whether different extra chromosomes vary in the amount by which they raise the induction rate of new interchanges.

The combination *velans·rubens lata*, of which several examples were examined, is indistinguishable in cytological behavior from *velans·gaudens lata*.

(ii) *gaudens·ʰblandina lata*. The extra chromosome was always independent of the ring of ten chromosomes of *gaudens·ʰblandina*. Instead it usually participitated in a trivalent with one of the two ring pairs. This group of three was present as a pair and a univalent in 4 cases, as a chain of three chromosomes in 5 cases, as a Y-trivalent or a ring-and-rod trivalent (figs. 10 and 11) in 13 cases, and as a triple arc (fig. 9) in 2 cases. In each of the configurations illustrated there is also a chain of ten chromosomes and a ring pair. The extra chromosome therefore has both pairing ends in common with the chromosomes of *gaudens* and *ʰblandina* making one of the two ring pairs (1·2 or 5·6). But in *velans·gaudens lata* it has been seen to be independent of 1·2 and therefore the *lata* chromosome is 5·6 from the *gaudens* complex.

(iii) *velans·flavens lata*. Figures 5–7 show three different types of critical configuration. Whereas diploid *velans·flavens* has two rings of four chromosomes and only three ring pairs, in figure 7 there are four ring pairs. This proves that the *lata* chromosome has both ends in common with one of the chromosomes by which *velans* and *flavens* differ. In figure 6, this particular pair of chromosomes is joined to a chain of three, and in figure 5 a ring of four chromosomes has a rod chromosome attached at one point by one end. The maximum configuration, with a ring pair closing the ends of a chain of three has been observed twice; in neither case could the object be drawn. Chromosome 5·6 is in *flavens*, but not in *velans;* the

observations are therefore compatible with the finding that the *lata* chromosome is 5·6.

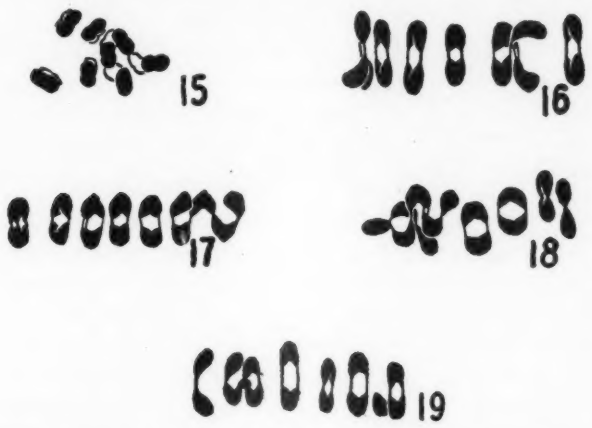


FIGURES 5–14. Metaphase I and diakinesis arrangements of chromosomes in pollen mother cells of various *lata* trisomics in progenies of *Oenothera Lamarckiana lata* pollinated by other interchange races. Figures 5–7 *velans·flavens lata;* figure 8 *velans·*^{h}*Hookeri lata;* figures 9–11 *gaudens·*^{h}*blandina lata;* figures 12–14 *velans·*^{h}*blandina lata.* ×4000.

(iv) *velans·*^{h}*Hookeri lata.* Figure 8 shows a diakinesis nucleus in which the extra chromosome is attached to a ring of four chromosomes. Other configurations observed include (i) a chain of five chromosomes with five ring pairs and (ii) a chain of three chromosomes with six ring pairs. The

extra chromosome is therefore identical with one of the four chromosomes (5·6, 6·7, 7·8, 8·5) normally forming a ring in *velans·ʰHookeri*.

(v) *velans·ʰblandina lata*. Three critical configurations are illustrated, namely, a chain of nine chromosomes and three pairs (fig. 12); a rod chromosome attached to a ring of eight chromosomes and three pairs (fig. 13); and a chain of seven chromosomes attached to a ring pair and three pairs (fig. 14). The extra chromosome is in the ring of eight of *velans·ʰblandina*, which contains chromosome 5·6 of *ʰblandina*.



FIGURES 15–19. Metaphase I arrangements of chromosomes in pollen mother-cells of *velans·ʰpurpurata lata* (figs. 15–17) and *velans·rubricalyx α lata* (figs. 18–19). ×4000.

(vi) *velans·ʰpurpurata lata*. In this combination the extra chromosome is identical with one of those in the ring of six which contains chromosome 5·6 of *ʰpurpurata*. In figure 15 there is a chain of seven chromosomes and four ring pairs; in figure 16 the chain of seven is replaced by chains of four and of three; and in figure 17 there is a chain of five chromosomes and five ring pairs.

(vii) *velans·rubricalyx α lata*. Figure 18 shows the extra chromosome attached to a chain of six chromosomes and figure 19 the presence of five ring pairs, one of them attached to a chain of two chromosomes. The extra chromosome is therefore identical with one of those in the ring of six chromosomes of *velans·rubricalyx α*, which contains the *rubricalyx α* 5·6 chromosome.

(b) Trisomics other than *lata*

(i) *Gaudens·ʰblandina w* had rather blunt pointed obovate lanceolate dark green, long-petioled rosette leaves. The cytological observations all

agree that the extra chromosome connects the ring of ten of *gaudens* ·^h*blandina* to one of the latter's free pairs (1·2 or 5·6). The other pair is still quite independent and must be 1·2, as both *velans* and *gaudens* have this chromosome. The extra chromosome therefore has the ends 5 or 6, but not both, as the second pair of chromosomes in *gaudens*·^h*blandina* is 5·6. Hence the extra chromosome is 5·8 or 6·7 from the *velans* complex.

(ii) *Velans*·^h*blandina y* occurred twice in the *Lamarckiana lata* by *blandina* families. The rosette leaves were rather narrow like those of *velans*·^h*blandina*, but darker green, more or less strongly convolute and with a very wavy margin and crinkled surface; the later stem leaves tended to be a much paler green. In both individuals the extra chromosome was independent of the ring of eight and connected together two of the three pairs (1·2, 3·4, 13·14) found in *velans*·^h*blandina*. Chromosome 1·2 is excluded for the same reason as before, hence the two chromosomes joined must be 3·4 and 13·14. The only possible chromosome that can do this is *gaudens* 3·13.

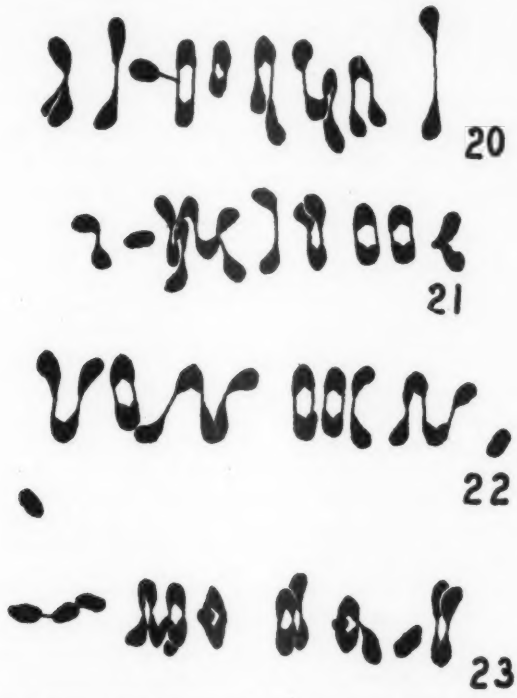
(iii) *Velans*·*blandina z* was of short stature, flowering when about a foot high. The leaves were broad, but more pointed, more crinkled and deeper green compared with those of *velans*·^h*blandina lata;* moreover the apex was not cucullate as in that type. The extra chromosome joined the ring of eight to one of the three free pairs (1·2, 3·4, and 13·14) of *velans* ·^h*blandina*. Since 1·2 is excluded as before, either 3·4 or 13·14 is the pair linked to the ring of eight chromosomes. The *gaudens* chromosomes 4·10 and 8·14 are the only ones capable of producing this configuration with *velans*·^h*blandina*.

Triploids

In two crosses in which the functional pollen of the male parent carried a curved stem complex, the only F_1 individuals surviving to maturity were tetrasomic triploids. Their sibs, 29 in number and all presumably diploid or trisomic, did not survive the cotyledon stage; they lacked chlorophyll and died. No triploids were found in other crosses; any present may have been passed over for trisomics.

(a) *velans*·*gaudens*·*undulans lata*, from *Lamarckiana lata*×*eriensis*, was rather short, had a curved stem and characteristics that could be ascribed to the *lata* chromosome. It had 22 chromosomes, that is, 3n+1. Side views of three typical metaphase I plates are shown in figures 20–22. No more than three ring pairs (fig. 22) have been seen in any one metaphase plate; twenty four complete metaphases have been examined for this point. Presumably two of the pairs represent the chromosomes 1·2, of *velans* and *gaudens*, and 5·6, of *gaudens* and *lata*. The third must therefore be due to a chromosome shared by *undulans* with either *velans* or *gaudens*.

(b) *velans·gaudens·divergens lata*, from *Lamarckiana lata* by *angustissima*, was very short, curved stemmed and had some characteristics of *lata;* it was 3n+1. Figure 23 shows a side view of metaphase I with the highest number of ring pairs, four, observed. Apart from 1·2 and 5·6, which account for two of these pairs, *divergens* must have two other chromosomes in common with *velans·gaudens*.



FIGURES 20–23. Metaphase I arrangements in pollen mother-cells of tetrasomic triploids. Figures 20–22 *velans·gaudens·undulans lata;* figure 23 *velans·gaudens·divergens lata*. ×4000.

DE VRIES (1913) has already used the survival of green triploids in the progenies of *Lamarckiana*, pollinated by *muricata*, *cruciata* and *Millersi*, to determine the frequency of unreduced megaspores in *Lamarckiana*. The last three species have pollen complexes which, with *velans* or *gaudens*, give white seedlings in the presence of *Lamarckiana* cytoplasm and plastids. Large numbers of plants can be handled easily, by counting the total seedlings and the number of green "Hero" (triploid) plants that survive. BRITTINGHAM and SHULL (1936) have evidently encountered the same

phenomenon in crosses of *O. Lamarckiana* and *O. franciscana* as egg parents with *O. Drummondii* as pollen parent In this case the progeny are predominantly green triploids with some pale green diploids. The triploids are ascribed to either double fertilization of the egg or to the production of embryos from the primary endosperm nucleus.

The two cases above in the *Lamarckiana lata* progenies render unlikely any explanation that is not based upon the functioning of unreduced eggs. The presence of the extra chromosome and the low frequency of ring pairs could not be accounted for otherwise.

SUMMARY

The extra chromosome in the dimorphic trisomic *Oenothera Lamarckiana lata* has been identified through a study of chromosome pairing at meiosis in various trisomic interchange combinations in which the *lata* chromosome was present as the extra one. In *O. Lamarckiana* (*velans·gaudens*) having chromosome 1·2 as a bivalent, the *lata* chromosome is one of the ring of twelve chromosomes. In *blandina·gaudens*, having chromosomes 1·2 and 5·6 as bivalents, the *lata* chromosome is homologous to the chromosomes of one bivalent and is independent of the ring of ten. It is therefore 5·6 and comes from the *gaudens* complex. The configurations in a series of six further different complex combinations with the *lata* chromosome added agree with this conclusion.

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LINKAGE GROUPS IN DROSOPHILA PSEUDOOBSCURA, RACE B

CATHERINE V. BEERS
University of Southern California, Los Angeles, California

Received June 25, 1937

INTRODUCTION

THE two races or physiological species, A and B, of *Drosophila pseudoobscura* offer a good opportunity for comparison of mutants, since they can be crossed reciprocally (LANCEFIELD 1925, 1929).

Through the work of METZ (1916), LANCEFIELD (1922, 1929), CREW and LAMY (1932, 1934, 1935, 1936), KOLLER (1932), DOBZHANSKY and STURTEVANT (Drosophila Information Service 1, 3, 7), TAN (1936), more mutants are known in race A than in race B. DONALD (1936) has summarized the linkage data for race A and has pointed out homologies between these chromosomes and those of *D. melanogaster*. TAN (1936) has revised the genetic maps of the autosomes of race A.

The present paper reports twenty new mutants in race B, distributed in four linkage groups. Six mutants are dominant, fourteen recessive. Twelve are sex-linked, eight autosomal. Four are autosomal dominants, lethal when homozygous, Smoky[1], Smoky,[2] Curly[1], Curly[2]. Two are sex-linked dominants which are lethal to all males carrying either of the Notch genes. One (beaded[1]) is a sex-linked recessive but appears only in the males. The females very seldom show beaded[1] even when homozygous. Nine mutants appeared in the descendants of irradiated wild type males. Two appeared in the descendants of wild type females caught in nature, and nine were observed during experimental counts or in routine stock observation.

Four mutants in race A are reported. Two autosomal recessives appeared in the second generation from females caught in nature. One of these, blister, has not been previously reported for race A. Two were observed during routine examination of stocks.

In race B, Smoky is a good workable dominant for linkage tests in the second group and Curly in the fourth. Only two recessives are known in the third group and none in the fifth. In the light of recent work on homologous genes it seems desirable to use names and symbols already established in race A (DONALD 1936). Therefore the name dela is changed to sepia (*se*). A summarized list of mutants, with origin, symbol, race and linkage group is given in table 1.

MATERIALS AND METHODS

The Hope, British Columbia, and La Grande (Washington) wild type stocks, and certain mutant stocks, were made available through the labo-

ratories at Columbia University and California Institute of Technology.

New wild type stocks of *pseudoobscura* were established from the offspring of females collected at Mammoth Creek, California, altitude 7,500 feet. The offspring of each female fertilized in nature were inbred in mass cultures to establish separate strains, identified as Mammoth Creek 1, 2, 3, etc.

TABLE 1

Summarized list of mutants in Drosophila pseudoobscura.

NAME	SYMBOL	CHARACTER AFFECTED	CHROMOSOME	DATE FOUND	STOCK
			Race B		
beaded[1]	b^1	wing margin	X	Apr. 23, 1934	Curly[2]/cinnabar
beaded[2]	b^2	wing margin	X	Nov. 29, 1935	scutellar sepia short
compressed	*co*	eyeless	X	May 3, 1936	singed bubble/scarlet
Curly[1]	Cy^1	wing	IV	Apr. 21, 1933	irradiated H., B.C. ♂
Curly[2]	Cy^2	wing	IV	Jan. 8, 1934	irradiated H., B.C., ♂
extended[1]	ex^1	wing position	autosomal	Dec. 8, 1931	irradiated H., B.C. ♂
extended[2]	ex^2	wing position	II	Jan. 4, 1932	irradiated H., B.C. ♂
Notch[1]	N^1	wing margin	X	Jan. 4, 1934	irradiated H., B.C. ♂
Notch[2]	N^2	wing margin	X	Jan. 6, 1934	irradiated H., B.C. ♂
scarlet	*st*	eye-color	X	Oct. 15, 1935	Notch[2]
scutellar	*sc*	bristles	X	Apr. 8, 1932	shortened/singed bubble
sepia (dela)	*se*	eye-color	X	Jan. 10, 1932	irradiated H., B.C., ♂
shortened	*sd*	5th longitudinal vein	III	Dec. 11, 1931	irradiated H., B.C. ♂
shortest	s^s	long. veins	X	Sept. 12, 1936	compressed/wild type
small bristle	*sb*	bristles	autosomal	Feb. 12, 1935	Mammoth Creek 7
Smoky[1]	Sm^1	veins	II	Apr. 30, 1933	La Grande 4
Smoky[2]	Sm^2	veins	II	Jan. 25, 1935	Mammoth Creek 10
vermilion	*v*	eye-color	X	Feb. 18, 1936	beaded[1]
vestigial	*vg*	wings	X	Feb. 13, 1932	irradiated H., B.C. ♂
white	*w*	eye-color	X	Feb. 10, 1936	irradiated M.C. 1 ♂
			Race A		
beaded[3]	b^3	wing margin	X	Aug. 30, 1935	Mammoth Creek 22b
blister	*bs*	wing	autosomal	Oct. 11, 1934	Mammoth Creek 8
purple	*pr*	eye-color	III	Oct. 11, 1934	Mammoth Creek 22
yellow	*y*	body color	X	Jan. 19, 1935	orange Scute purple

In order to obtain more mutants for use in mapping race B chromosomes, males from wild type stocks, Hope and Mammoth Creek, were irradiated and their inbred progeny observed.

The flies were raised at room temperature below 24°C on the Bridges Standard cornmeal molasses food formula.

THE X CHROMOSOME OF RACE B

The construction of the genetic map

From the standard data included in tables 2 to 6 a genetic map for the X chromosome of race B has been constructed (fig. 1). Linkage tests have

been made for twelve mutants and the primary recombination percentages have been used to indicate that the total length is about 165 units. It is

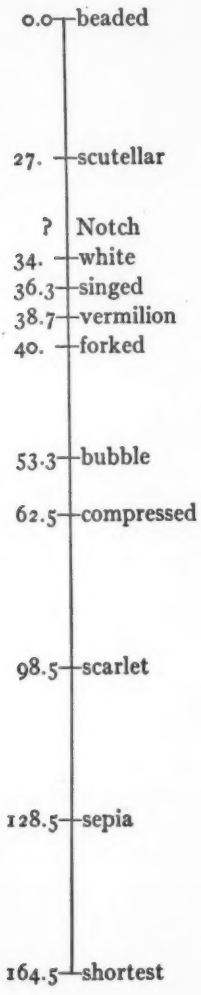

FIGURE 1.

obvious that this is a preliminary map. The distances between genes, and consequently the total length, will probably be increased as linkage data for new mutants are made available.

TABLE 2

Two point linkage experiments.

LOCI*	TYPE OF CROSS	NON CROSSOVERS		CROSSOVERS		TOTAL
bu co	*bu*×*co*	552	360	17	76	1,005 ♂
b[1] *st*	*b*[1]×*st*	25	42	21	38	126 ♂
sc se	*sc*×*se*	758	756	727	750	2,911 ♂
sn co	*sn co*×+	82	131	43	33	288 ♂

* Loci are listed beginning with the left-most mutant.

TABLE 3

Three point linkage experiments.

TYPE OF CROSS	NON CROSSOVERS		SINGLE CROSSOVERS REGION 1*		SINGLE CROSSOVERS REGION 2		DOUBLE CROSSOVERS		TOTAL
b[1]×*sc se*	37	33	3	10	19	24	4	14	144 ♂
b[1]×*sn bu*	30	24	8	15	0	3	15	1	96 ♂
co st×*s*+	52	204	151	87	61	182	110	97	944 ♂
N[1]×*sn bu*	112	111	0	0	1	28	0	0	252 ♀
N[1]×*sn bu*	1,942	1,824	0	1	44	250	2	0	3,963 ♀
N[2]×*sn bu*	1,909	1,743	6	11	90	433	6	11	4,209 ♀
sc bu×*se*	213	259	53	33	168	110	43	26	1,005 ♂
sc se×*N*[1]	187	137	16	3	144	111	15	1	614 ♀
sc f×*v*	352	278	22	15	3	4	0	0	674 ♂
sc f×*w*	526	455	40	43	27	30	0	0	1,121 ♂
sn bu×*st*	134	139	30	21	74	98	28	1	525 ♂
sn bu×*se*	91	86	15	8	71	60	8	6	345 ♂
sn co×*st*	615	779	91	79	92	329	25	1	2,011 ♂
w×*sn bu*	563	608	5	16	56	167	2	1	1,418 ♂
w sn×*v*	188	207	3	9	0	5	0	0	412 ♂

* Both complementary classes are conventionalized by putting first the class starting with the left-most mutant.

TABLE 4

Four point linkage experiments.

	TYPE OF HYBRID FEMALE		
	b sc se s	*sc f se* / *N*[2]	*sc sn bu* / *se*
0	293+307	137+142	391+460
1	104+ 86	4+ 4	58+ 35
2	299+274	1+ 5	42+ 21
3	155+175	140+194	338+386
1, 2	93+ 94	0+ 0	2+ 22
1, 3	63+ 64	1+ 4	48+ 15
2, 3	174+156	0+ 6	46+ 14
1, 2, 3	55+ 46	3+ 2	2+ 9
Total	2,438 ♀	643 ♀	1,889 ♂

Beaded wing is the known mutant at the extreme left and is therefore taken as the zero point. There are two alleles of beaded. Beaded[1] shows very well in the males but not in the females. Beaded[2] is expressed in males and females. Scutellar is 27 units to the right of beaded. The region of 26.3 units between scutellar (27) and bubble (53.3) is marked by six mutants; Notch[1], Notch[2], white, singed, vermilion, and forked. This group is near the middle of the chromosome in race A. Bubble is in the right limb and a direct test gave 9.2 percent of recombination between bubble

TABLE 5

Five point linkage experiments.

	TYPE OF HYBRID FEMALE	
	b sc se	*b sc se s*
	sn bu	*st*
0	93+103	113+141
1	14+ 32	49+ 40
2	5+ 17	92+ 82
3	3+ 10	56+ 48
4	112+ 89	77+ 65
1, 2	3+ 2	39+ 28
1, 3	31+ 6	19+ 23
1, 4	12+ 42	26+ 26
2, 3	7+ 1	37+ 50
2, 4	5+ 17	47+ 63
3, 4	2+ 12	28+ 23
1, 2, 3	0+ 2	30+ 18
1, 2, 4	3+ 2	33+ 24
1, 3, 4	32+ 8	9+ 8
2, 3, 4	11+ 0	22+ 22
1, 2, 3, 4	0+ 1	12+ 4
Total	677 ♂	1,354 ♂

and compressed. A count of 288 ♂♂ gave 26 percent of recombination between singed and compressed. This figure will place compressed to the right of bubble which coincides with the sequence in race A. Further experiments are necessary to settle definitely this sequence in race B.[1] Three other mutants widely separated in the right limb are scarlet at 98.5, sepia at 128.5, and shortest at the end of the map at 164.5. Beaded in the left, white, singed and vermilion near the middle region, compressed, sepia and shortest in the right limb were found to be allelic markers for the two races.

The Notch region

Since both of the Notch mutants are dominant in the females and all of the males which carry either Notch deficiency die it is not possible to

[1] Since this paper went to press a count of 1312 ♂♂ verifies this sequence in Race B.

prove by direct breeding methods that the two mutants are alleles. Comparative linkage data were obtained from females heterozygous for Notch and singed bubble. In a count of 3,963 females, Notch[1] gave 0.07 percent of recombination with singed and in 4,209 females, Notch[2] gave 0.8 percent of recombination with singed. These figures suggest an identical locus

TABLE 6

Summarized data for each pair of loci in the X chromosome.

REGION*	TOTAL	CROSSOVERS	PERCENTAGE OF CROSSING OVER
b[1] *sc*	144 ♂	31	21.
b[1] *sn*	96 ♂	39	40.
b[1] *st*	126 ♂	59	46.
b[2] *sc*	4,469 ♀	1,183	27.
sc N[1]	614 ♀	35	5.
sc w	1,121 ♂	83	7.4
sc sn	2,566 ♂	267	10.
sc v	674 ♂	37	5.5
sc bu	1,005 ♂	155	15.
sc st	1,354 ♂	603	43.
sc se	5,493 ♂	2,729	48.
N[1] *sn*	4,215 ♀	3	0.07
N[2] *sn*	4,209 ♀	34	0.8
N[2] *f*	643 ♀	16	2.4
w sn	1,830 ♂	36	1.9
w f	1,121 ♂	57	5.1
v f	674 ♂	7	1.4
sn v	412 ♂	10	2.4
sn bu	2,716 ♂	451	17.
sn co	288 ♂	76	26.
bu co	1,005 ♂	93	9.2
bu st	525 ♂	201	38.
bu se	3,916 ♂	1,798	45.
co st	2,955 ♂	1,076	36.
st se	1,354 ♂	409	30.
st s[a]	944 ♂	558	59.
se s	3,792 ♂	1,377	36.

* The data used in the above table are composed entirely of data in which no intermediate locus was present between the loci concerned.

for the two Notch mutants. In the count of 3,963, the *N*[1]-*bu* and the *sn*-*bu* recombination percentages were both 4.9 percent; while in the 4,209 female count the *N*[2]-*bu* and the *sn*-*bu* recombinations were both 12 percent. Both the 4.9 percent for *N*[1] and the 12 percent for *N*[2] were based on the crossing over in females heterozygous for Notch and singed bubble. The singed bubble flies came from the same stock in both experiments so the difference is evidently in the two Notch cultures. Neither Notch is a deficiency for the singed or white locus. In a count of 588 testcross females no recombina-

tion was obtained between Notch and white. Notch is placed to the left of white but the exact locus is unknown.

The recombination percentage of the scutellar-forked interval when Notch^2 was present in the opposite chromosome was 2.8 in a count of 643 females. But when Notch was omitted and the test crosses involved *sc*, *w*, *sn*, *v*, and *f* the sum total of these recombination percentages is 12.1 which is comparable to the distance in race A (DONALD 1936b; STURTEVANT and TAN, Drosophila Information Service 7). Notch^2 has a reducing effect on crossing over in this region of the X chromosome.

Allelic genes in the two races

Two beaded mutants have been tested in each race. In race A the beaded miniature sepia snapt stock and a new beaded from M.C.22 were used. The test made was b♂(A)$\times b^2$♀(B) and it produced b(A)/b^2(B) females which appeared beaded. When using beaded^1(B) the test was not so direct because the females seldom have beaded wings. The cross b^1(B)$\times b^2$(B) produced females whose sons all had beaded wings. The mutants white, singed, vermilion, compressed (eyes usually entirely absent), sepia and shortest (more extreme in males than in females) were shown by direct mating tests to be alleles of white-eosin, singed, vermilion, compressed, sepia, and short of race A. Only these seven mutants were available in race A for tests at this time.

Comparison of the linkage maps in race A and race B

The linkage map of the X chromosome of race A (DONALD 1936b; STURTEVANT and TAN, Drosophila Information Service 7) is longer than the present map of race B. In race A, Pointed is 21.9 units to the left of beaded and at the other end tilt is 2.3 units to the right of short. Also more intermediate loci have been observed. These facts tend to increase the race A map distance.

The sequence of the loci which are known on both maps is identical. LANCEFIELD (1929) and KOLLER (1932) reported normal crossing over in the middle region of the X chromosome of the A/B hybrids. That is to be expected with the identical sequence of mutants near the middle region recorded in this paper. They further reported that suppression of crossing over occurred in both ends. TAN (1935), KOLLER (1936), DOBZHANSKY and TAN (1936) observed cytological inversions in both ends. These inversions should be apparent in the genetic map but there are relatively long distances in both limbs which are as yet unmarked in race B. These regions, therefore, probably include the expected inversions.

AUTOSOMAL LINKAGE GROUPS

There are four pairs of autosomes in race B as in race A. Three of these

contain six new genes but there is not sufficient data at present to determine the sequence of the loci.

Smoky[1], Smoky[2], and extended[2] are linked with cinnabar, which is linked with the Bare-Minute of race A and are therefore placed in the second linkage group (DOBZHANSKY 1936, DONALD 1936b, TAN 1936).

Shortened is linked with orange in the third linkage group.

Curly[1] and Curly[2] are not linked with either of the other two groups and are therefore placed in the fourth group.

Group five of race B is still to be marked.

RECURRENCE OF MUTATIONS

Certain of the genes appear to mutate more frequently than others. In this study of race B five of the mutants have appeared more than once. The possibility of contamination has been eliminated. Notch has been found four times, Smoky and Curly each twice, and the two recessives beaded and extended each twice. In the race A stocks, yellow, beaded, and purple alleles have appeared.

AN EXAMINATION OF EIGHTEEN STRAINS COLLECTED IN NATURE

A collection of eighteen females fertilized in nature was made on Mammoth Creek, California, August 1934. By crosses with Pointed (A) and Curly (B) five strains proved to be race A and thirteen race B. M.C.2(A) and 8(A) have the Y chromosome of type IV. M.C.1(B) has type I and M.C.10(B) has the Y of type III (DOBZHANSKY 1935). None of the original flies showed any abnormalities. The F_1, F_2 and in some cases later generations were examined; a few individuals with wings notched or cut were found in two strains and with blister or bubble in seven strains. No visible mutant was found in the thirteen strains of race B during the first three generations. During routine stock examination in the fifth month Smoky[2] was found and in the sixth month several flies with small bristles, but they did not produce a stock. Two mutants were found in the five strains of race A. M.C.8(A) produced an autosomal recessive blister of good viability but not 100 percent expression. In the F_2 of M.C.22(A) purple males and females were found and when mated to orange Scute purple stock produced all purple offspring. Dwarf has also appeared sporadically in M.C.22. A sex-ratio gene was evidently present in M.C.22(A) and M.C.13(B) which causes the male that carries it to produce nearly all X sperm instead of the usual 50 percent (STURTEVANT and DOBZHANSKY 1936). GORDON (1935) reported that 23 wild strains of *Drosophila melanogaster* gave 14 mutants, and in *D. subobscura*, 26 strains gave 24 mutants.

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The research was carried on at Columbia University and at the University of Southern California.

SUMMARY

1. Four linkage groups of race B have been identified by marking genes. Groups two and four each contain a good workable dominant. Group three has two recessives and group five is not marked.

2. Twelve mutants were used in the construction of the linkage map of the X chromosome of race B (fig. 1).

3. The sequence of the known loci in race B is identical with the map of race A.

4. Seven mutants in race B have alleles in race A.

5. Mutations of five genes appeared more than once in this study of race B.

6. A new autosomal blister is reported in race A.

7. Thirteen wild strains of race B gave no visible mutant in three generations and five wild strains of race A gave three mutants.

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DEVELOPMENT OF EYE COLORS IN DROSOPHILA: FAT BODIES AND MALPIGHIAN TUBES IN RELATION TO DIFFUSIBLE SUBSTANCES

G. W. BEADLE[1]
Harvard University, Cambridge, Massachusetts

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IT HAS been shown that diffusible substances are involved in the differentiation of certain eye colors in *Drosophila melanogaster* (EPHRUSSI and BEADLE 1937a; BEADLE and EPHRUSSI 1937). Fat bodies are concerned with the production of one of these substances (v^+ substance); two substances (v^+ and cn^+ substances) can be obtained from the Malpighian tubes of flies of certain genotypes. Preliminary experiments having to do with fat bodies and Malpighian tubes have been reported (BEADLE 1937a). It is the purpose of this paper to report more extensive studies of the relation of fat bodies and Malpighian tubes to eye color development. In particular, tests of fat bodies and Malpighian tubes of various eye color mutant types are reported. Certain experiments have been directed toward determining whether Malpighian tubes produce the substances obtainable from them or, on the other hand, whether these substances are produced in other parts of the body and are merely taken up by Malpighian tubes. Certain results bear on the question whether flies of certain constitutions, cinnabar for example, are characterized by complete absence of certain diffusible substances, cn^+ substance in the case of the cinnabar fly, or merely by what might be called sub-threshold concentrations.

MATERIAL AND METHODS

In the experiments reported here, most of the known eye color mutants were used. A list of these will be found in BEADLE and EPHRUSSI (1936a). In addition to the mutants listed in the above paper, certain experiments involve the mutants bright (*bri*), mahogany (*mah*), and suppressor of vermilion (*su-v*). An Oregon-r wild type stock was used.

All experimental animals were grown at a temperature of approximately 25°C.

The method of transplanting fat bodies and Malpighian tubes used was essentially that described by EPHRUSSI and BEADLE (1936). In the case of fat bodies, relatively large-bore, long-shaft pipettes were used. Unless otherwise designated, animals were taken for operations shortly before puparium-formation, that is, about three days after hatching from the eggs.

[1] Now at Stanford University, California.

Vermilion brown and cinnabar brown larvae were used in testing for the presence of, or production of, active substances, the former for v^+ substance, the latter for cn^+ substance. Results were recorded in a roughly quantitative way as described briefly by THIMANN and BEADLE (1937). In the arbitrary scale of color intensities used, 0 represents no effect and 5 represents the maximum possible effect or an eye color not distinguishable from that of brown flies of comparable age. It should be emphasized that the eye color values recorded are not necessarily strictly comparable unless specifically stated to be so. A color-value of, for example, 3 recorded at one time may not represent exactly the same modification as the same numerical value recorded at a different time. This difficulty could be largely avoided if a series of standard stocks with eye colors of various degrees of intermediacy between cinnabar brown (practically white and lighter than vermilion brown) and brown were used. Such a series of "standards" has not yet been developed.

Extracts of Malpighian tubes, referred to as "Ringer-extracts," were made by dissecting larvae, usually five at a time, and then transferring the tubes to known volumes of Ringer's solution in capillary tubes. After each set of five dissections the capillary tube was heated by holding it for a short time in boiling water. Finally the Malpighian tubes were thrown to the bottom by centrifuging and the clear extract drawn off with the micro-pipette used for injection. The number of larvae into which the total volume of extract was put was recorded; the volume of extract injected per larva is therefore approximately known. For injecting fluid, pencil-point micro-pipettes were used. With proper precautions, little liquid need be lost, though it is never possible to be absolutely certain that all of the injected liquid remains in the recipient.

Since preliminary experiments had indicated little or no difference between male and female donors of fat body or Malpighian tube transplants, no attempt was made to obtain the four possible sex-combinations in each series. In some cases larvae of one sex only were selected as donors, in other experiments donors were selected at random with respect to sex. In the preparation of extracts of various kinds, male and female donors were used indiscriminately. In all tables, under the heading "number of individuals" the order of recording of recipients is: female, male, and total.

FAT BODIES AND v^+ SUBSTANCE

If a portion of the fat body of a wild-type larva is transplanted to a vermilion brown larva, the latter develops an eye color strongly modified in the direction of brown (BEADLE 1937a). It has been shown that essentially the same result is obtained when such transplants are made about forty hours before puparium-formation as when they are made shortly

before puparium-formation. Fat bodies from male and female donors are equally effective in bringing about this modification. The simultaneous transplantation of an ovary seems to make no difference. Various sections of the fat body are effective; there is no indication of regional differences.

Tests of fat bodies of various eye color mutants

Tests of fat bodies of the various eye color mutants were made by transplanting the greater part of one lateral fat body to vermilion brown larvae. The results of such tests are summarized in table 1. Positive tests were obtained in all tests except those of fat bodies of vermilion and suppressor of vermilion, vermilion. It is seen that the mean numerical values of the tests vary considerably. This may be the result of differences in amount of v^+ substance produced by different fat bodies, but this is not at all certain for several reasons. In the first place, it is difficult to be sure that the amount of fat body transplanted is the same in different tests. Secondly, there is no way of being certain that the entire piece transplanted remains in the recipient. Lastly, there is a certain variability in scoring the various tests. In two instances (wild and *pr*), two series of tests are given in table 1 for a single type of fat body. In these cases, the tests were made at different times and the results are not strictly comparable because of possible differences in scoring. In both pairs of tests, there are rather wide differences. In the first test of purple, a very low value was recorded (0.9). In order to see whether or not this particular low value was of significance, tests for purple were repeated and at the same time tests of wild type and cinnabar brown fat bodies were tested. Care was taken to transplant as nearly the same amounts of fat body in all of these tests and at eclosion the recipients were compared carefully. These tests, indicated in the footnote to table 1, gave values very close to one another, 1.9, 1.8 and 1.9 respectively. In these cases, then, there is no indication of significant differences in the amount of v^+ substance produced.

Attempts to extract v^+ substance from fat bodies

Since it it known that v^+ substance is water-soluble (KHOUVINE, EPHRUSSI and HARNLY 1936; THIMANN and BEADLE 1937) and can readily be extracted from Malpighian tubes with hot Ringer's solution (BEADLE 1937a), attempts were made to extract this substance from fat bodies. Fat bodies were removed, placed in capillary tubes in Ringer's solution and broken up by heating and crushing. After centrifugation, a somewhat turbulent supernatant liquid was obtained. Injected into vermilion brown larvae, this gave negative results. Several extraction experiments are summarized in table 2. These unsuccessful attempts to extract v^+ substance from fat bodies indicate that v^+ substance is not produced

until after puparium formation. It is possible, of course, that the extraction method was inadequate, but this seems rather unlikely since it is known to work for Malphigian tubes. Another possibility is that the substance is released immediately on formation and consequently never reaches

TABLE 1

Effects of fat body transplants of various genotypes on the eye color of vermilion brown hosts.

IMPLANT‡	NUMBER OF INDIVIDUALS			NUMBER OF NEGATIVE TESTS	EYE COLOR OF HOSTS, MEAN AND RANGE
	♀	♂	Total		
wild	7	3	10	0	3.5 (3.0–4.0)
wild	3	1	4	0	1.8*(0.3–2.7)
bo	4	5	9	0	2.5 (2.0–3.7)
bri	1	1	2	0	3.2 (2.7–3.7)
bw	5	11	16	0	2.2 (1.0–3.0)
car	2	5	7	0	3.0 (2.7–3.7)
cd	4	6	10	0	2.4 (0.7–4.0)
cl	6	8	14	0	3.0 (1.7–4.0)
cm	3	5	8	1	1.9 (1.0–3.0)
g^2	6	2	8	0	4.0 (3.3–5.0)
Hn^r	1	4	5	1	2.4 (2.3–2.7)
lt	1	2	3	0	4.2 (4.0–4.3)
ma	8	6	14	0	3.7 (3.3–4.0)
mah	3	2	5	0	2.3 (2.3)
p^p	4	4	8	0	2.4 (1.3–4.0)
p^d	5	1	6	0	1.4 (1.0–2.3)
p^n	7	2	9	0	2.8 (2.3–3.3)
pr	3	7	10	1	0.9 (0.3–1.7)
pr	5	3	8	0	1.9*(1.3–2.7)
ras^2	3	5	8	0	2.5 (1.0–3.3)
rb	3	6	9	0	3.2 (1.3–4.0)
se	6	5	11	0	2.8 (2.0–3.3)
sed	5	6	11	0	2.2 (1.3–3.3)
sf^2	9	4	13	0	1.6 (0.7–2.3)
st	3	3	6	0	3.1 (2.0–3.7)
v	3	5	8	8	0.0 (0.0)
w	2	2	4	1	2.1 (2.0–2.3)
cn bw	2	2	4	0	1.8*(0.7–2.3)
su-v v	6	4	10	10	0.0 (0.0)

‡ Results of *ca* and *cn* transplants published in a previous paper (BEADLE 1937a).

* The indicated values for wild, *pr*, and *cn bw* are comparable in that the transplants were made at the same time and the hosts directly compared with one another.

a concentration in the fat body cell sufficiently high to give a test on extraction; this does not seem very probable.

FAT BODIES AND cn^+ SUBSTANCE

In contrast to results obtained in tests for v^+ substance, wild type fat bodies transplanted to cinnabar brown flies produce no modification of the

eye color (BEADLE 1937a). Evidently fat bodies do not produce cn^+ substance. In order to see whether this inability of the fat body to produce cn^+ substance might possibly be a peculiarity of certain genetic types,

TABLE 2

Test of Ringer-extracts of larval fat bodies for effects on the eye color of vermilion brown and cinnabar brown recipients.

DONORS	RECIPIENTS	NUMBER SETS FAT BODIES EXTRACTED	RINGER'S USED (cc)	NUMBER LARVAE INJECTED	NUMBER OF INDIVIDUALS			EYE COLOR RECIPIENTS
					♀	♂	Total	
wild	*v bw*	?	?	?	11	7	18	0.0
wild	*v bw*	30	0.03	33	8	9	17	0.0
wild	*cn bw*	?	?	?	5	10	15	0.0
wild	*cn bw*	30	0.03	33	3	9	12	0.0
bri	*v bw*	30	0.02	11	4	6	10	0.0

tests were made of fat bodies of ten additional eye color mutants (claret was previously tested, BEADLE 1937a). All tests were clearly negative (table 3). Ringer-extracts of wild type fat bodies likewise give negative

TABLE 3

Tests for effects of fat body transplants of various genotypes on the eye color of cinnabar brown hosts.

IMPLANT‡	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS	IMPLANT	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS
	♀	♂	Total			♀	♂	Total	
bo	3	1	4	0.0	Hw^r	0	1	1	0.0
bw	3	4	7	0.0	*lt**	6	3	9	0.0
cl	2	4	6	0.0	p^p	4	3	7	0.0
cm	2	3	5	0.0	*rb*	4	6	10	0.0
g^2	5	4	9	0.0	*w*	3	6	9	0.0

‡ Results of wild type and *ca* transplants published in a previous paper (BEADLE 1937a).
* Donors male

results in tests for cn^+ substance (table 2). It is believed that a sufficiently large sample of genetic types have been tested to indicate rather strongly that cn^+ substance is never produced by fat bodies.

MALPIGHIAN TUBES AND DIFFUSIBLE SUBSTANCES

As shown by transplants made in the late larval stage, wild type Malpighian tubes produce, or at least release, both v^+ and cn^+ substances (BEADLE 1937a). A general survey of the release of these substances by Malpighian tubes of the various eye color mutants has been made and is presented below.

Comparison of different regions of Malpighian tubes

In *Drosophila melanogaster* there are four Malpighian tubes, an anterior and a posterior pair. Judging from general observations made during dissections of larvae of various genetic stocks, the size of the Malpighian tubes relative to larval size varies in larvae of different genetic constitutions. In some stocks the tubes are long and slender while in others they are relatively shorter and thicker. Presumably these relations are genetically controlled. Measurements of the posterior tubes of larvae of the sepia stock taken shortly before puparium formation showed that these tubes were about 3.2 mm long and about 0.4 mm in diameter at the mid-region. The anterior tubes are generally about 1.2 to 1.5 times the length of the posterior tubes. In diameter both anterior and posterior tubes are largest at their bases; both gradually taper toward their distal ends, the posterior tubes to the end and the anterior tubes to a point lying approximately in the mid-section. The anterior tubes of wild type larvae are obviously differentiated into two regions. The proximal regions are similar to the corresponding regions of the posterior tubes. The distal regions of the anterior tubes, one third to one half the total length, are white as contrasted with the yellow proximal regions, are generally more or less swollen, apparently relatively thin-walled, and have their lumens filled with white material—calcium carbonate according to the literature.

A large number of comparisons have been made of the release of v^+ and cn^+ substances by anterior and posterior tubes. In practically all tests recorded in tables 5 and 6, the two pairs were kept separate. The preliminary comparisons indicated a somewhat greater effect of the anterior pair (BEADLE 1937a), but examination of a large number of comparable tests showed little or no difference. In tabulating the data, therefore, the two pairs are listed together.

Comparisons of the yellow proximal regions of the anterior pair of tubes with the white distal regions of the same tubes in tests for both v^+ and cn^+ substances are summarized in table 4. In this table the tests within a given group, indicated by spacing, are strictly comparable. In these, as in all tests of Malpighian tubes made, presence of the implant at eclosion was determined by dissection. It is evident that the greater part, probably all, of the activity in both tests is confined to the yellow proximal region.

Tests of separate regions of the posterior pair of tubes are given in table 4. Tests for cn^+ substance only were made. The proximal and distal halves have approximately equal activities. Approximate separation into proximal one-tenth and distal nine-tenths show, as expected from the above, a large difference. There is, then, no indication of regional differences in activity in the posterior pair of tubes.

Malpighian tubes of various mutants and v⁺ substance

The effects of Malpighian tube implants of various genetic types on the eye color of vermilion brown hosts are indicated in the tests summarized in table 5. In tests of Malpighian tubes two sources of variability, difficult to control in tests of fat bodies, are avoided. In the first place, entire Malpighian tubes can be transplanted and it is therefore possible to be reasonably certain that different tests are approximately comparable in so far

TABLE 4

Comparison of the effects of different regions of wild type Malpighian tubes on the eye color of vermilion brown and cinnabar brown hosts. Female donors.

IMPLANT	HOST	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
		♀	♂	Total	
Yellow proximal regions of anterior pair	*v bw*	3	4	7	4.0 (3.3–4.7)
White distal regions of anterior pair	*v bw*	2	4	6	0.0
Entire posterior pair	*v bw*	1	3	4	4.0 (4.0)
Yellow proximal regions of anterior pair	*v bw*	3	1	4	3.3 (3.3)
White distal regions of anterior pair	*v bw*	2	3	5	0.0
Yellow proximal regions of anterior pair	*cn bw*	4	6	10	3.1 (1.3–4.0)
White distal regions of anterior pair	*cn bw*	5	4	9	0.0
Proximal one-half of posterior pair	*cn bw*	6	5	11	1.8 (0.7–2.7)
Distal one-half of posterior pair	*cn bw*	5	3	8	2.0 (0.7–3.0)
Proximal one-tenth of posterior pair	*cn bw*	1	5	6	0.8 (0.3–1.3)
Distal nine-tenths of posterior pair	*cn bw*	6	3	9	2.6 (0.7–3.7)

as the amount of material transplanted is concerned. Secondly, implants can be recovered in the adult flies and all individuals on which the transplantation operation was not completely successful can be discarded. It is clear from table 5 that, notwithstanding these precautions, there is a good deal of variability in different tests. Some of this undoubtedly results from differences in evaluating color-grades in tests made at different times. There remain, however, differences which cannot be accounted for on this basis. These will be considered in a later section of this paper.

Malpighian tubes of various mutants and cn⁺ substance

Tests, comparable to those for v^+ substance given above, for cn^+ substance are presented in table 6. Here again there are differences that cannot be accounted for by differences in evaluation of color-grades. The only negative test for cn^+ substance recorded is that of tubes of cinnabar larvae; this of course is in agreement with our previous interpretation of the relation of diffusible substance to eye color differentiation.

TABLE 5

Effects of Malpighian tube transplants of various genotypes on the eye color of vermilion brown hosts. Anterior and posterior tubes are listed together.

IMPLANT‡	SEX OF DONOR	NUMBER OF TUBES TRANSPLANTED	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
			♀	♂	Total	
wild	♀, ♂	2	11	6	17	3.2 (2.0–4.0)
bo	♀	2	5	5	10	3.5 (3.0–3.7)
bw	♀	2	3	6	9	2.8 (2.2–3.2)
bw	♂	2	3	7	10	2.0 (0.7–2.7)
car	♀	2	3	5	8	1.1 (0.7–2.0)
car	♀	2	6	8	14	2.0 (0.7–2.7)
cd	♀	2	8	3	11	3.2 (2.7–3.7)
cl	♀	2	6	7	13	2.4 (0.7–3.7)
cm	♀	2	2	3	5	2.6 (2.0–3.0)
cn	♀	4	6	2	8	2.5 (2.3–3.0)
g^2	♀, ♂	2	4	5	9	1.1 (0.7–1.7)
g^2	♀	2	5	0	5	1.0 (0.3–1.7)
Hn^r	♀	2	6	10	16	2.4 (1.0–3.3)
lt	♀	2	7	7	14	0.0 (?)
ma	♀	2	7	6	13	2.6 (1.0–3.7)
mah	♀	2	4	7	11	3.5 (3.0–4.3)
p^p	♀	2	6	3	9	0.0 (?)
pd	♀	2	8	7	15	2.7 (2.0–3.3)
pn^2	♀	2	6	5	11	2.5 (2.0–3.7)
pr	♀	2	4	7	11	2.4 (1.0–3.0)
ras^2	♀	2	7	3	10	3.1 (2.0–4.3)
ras^2	♂	2	4	5	9	3.7 (2.0–4.3)
rb	♀	2	4	5	9	2.8 (2.0–3.0)
se	♀	2	3	6	9	2.9 (1.3–4.0)
sed	♀	2	10	2	12	1.9 (0.3–3.0)
sf^2	♀	2	7	5	12	3.2 (2.0–4.3)
st	♀	2	7	6	13	0.0
st	♀	2	9	6	15	0.0
st	♀	4	4	2	6	0.0
v	♀	2	10	10	20	0.0
w	♀	2	6	2	8	0.0 (?)
su-v v	♀	2	6	3	9	0.0 (?)
su-v v	♀	4	4	3	7	0.3 (0.0–0.4)

‡ Tests of wild type (additional), *bri*, *ca*, and *cn* (2 tubes) published in a previous paper (BEADLE 1937a).

Relation between Malpighian tube color and release of substances

As previously pointed out (BEADLE 1937a), there appears to be a general relation between intensity of pigmentation of Malpighian tubes and their effects, when transplanted, on the eye color of vermilion brown and cinnabar brown hosts.

TABLE 6

Effects of Malpighian tube transplants of various genotypes on the eye color of cinnabar brown hosts. Two tubes, anterior or posterior, transplanted in each test.

IMPLANT*	SEX OF DONOR	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
		♀	♂	Total	
wild	♀	2	5	7	4.2 (3.2–4.8)
bo	♀	3	10	13	3.3 (2.0–4.0)
bri	♀	4	8	12	0.8 (0.0–1.3)
bw	♀	4	7	11	2.4 (1.0–3.3)
car	♀	4	8	12	1.5 (1.0–2.3)
car	♂	4	4	8	1.0 (0.7–1.3)
cd	♂	6	4	10	3.0 (1.0–4.0)
cl	♀	10	4	14	2.6 (1.0–4.0)
cm	♀	3	5	8	2.7 (1.7–3.3)
cn	♀	5	7	12	0.0
g^2	♀, ♂	6	1	7	0.9 (0.7–1.0)
g^2	♀	3	3	6	0.4 (0.3–1.0)
Hn^r	♀, ♂	7	10	17	2.2 (1.0–3.0)
mah	♀	6	5	11	3.1 (1.0–4.0)
p^p	♀	6	4	10	0.6 (0.0–1.0)
p^d	♀	1	4	5	2.8 (2.7–3.0)
pn^2	♀	5	11	16	2.4 (1.0–3.7)
pr	♀	7	6	13	2.3 (1.7–2.7)
ras^2	♀	5	8	13	3.5 (0.7–4.7)
ras^2	♀	8	6	14	2.4 (1.0–3.3)
rb	♀	4	4	8	2.4 (1.8–3.0)
se	♀	7	2	9	2.3 (0.7–3.3)
sed	♀	7	4	11	2.1 (0.7–3.3)
sf^2	♀	5	5	10	2.9 (2.3–3.7)
st	♀	1	7	8	0.6 (0.3–0.7)
st	♀	9	8	17	0.3 (0.1–0.6)
v	♀	3	3	6	0.4 (0.3–0.7)
v	♀	3	3	6	0.4 (0.3–0.7)
su-v v	♀	8	7	15	0.4 (0.0–2.3)

* Tests of wild type (additional), *ca*, *lt*, *ma*, and *w* published in previous paper (BEADLE 1937).

The eye color mutants can be grouped, according to larval Malpighian tube color, as follows (see also BEADLE 1937b): bright yellow (approximately the same as wild type), wild type, *bo*, *cd*, Hn^r, *mah*, *pd*, pn^2, *se*, *sed*, and sf^2; pale yellow, *bri*, *bw*, *car*, *cn*, *ma*, *pr*, *st*, and *v;* very pale yellow, color largely confined to base of tubes, *cm*, g^2, *rb;* white, *ca*, *lt*, p^p, and *w*. Examinations of the tests of tubes of the first group (bright yellow) show that they give relatively strong effects, generally color values of two or more (tables 5 and 6). Whether the differences recorded within this group have any real significance is not known—there can be a reasonable doubt.

Tubes from larvae of the second group give rather variable results. Cinnabar gives a medium test for v^+ substance, none for cn^+ substance, scarlet and vermilion tubes give negative results for v^+ substance and very weak tests for cn^+ substance. Tubes of the third group (very pale yellow with color confined to base of tubes) give variable results, garnet being relatively weak in both, carmine and ruby being relatively strong. The third group gives very weak tests for v^+ substance (close to o) and somewhat stronger tests for cn^+ substance (see BEADLE 1937a for tests not recorded in tables 5 and 6).

It is seen from the above that the relation between Malpighian tube color and activity in tests for diffusible substances is in no sense an absolute one. Tubes as deeply pigmented as those of wild type all seem to be rather high in activity. Pale yellow tubes, however, appear to be almost as active in some cases. On the other hand, pale yellow tubes may have very little or no activity in the tests. White tubes have very little activity.

It is evident that some developmental relation exists between the pigment system in the eye and that in the Malpighian tubes. Just what this relation is, the available information does not tell us. It is not a simple qualitative relation between amount of one or both diffusible substances produced and the intensity of pigmentation of the Malpighian tubes. Further, there does not seem to be a linear relation between the amount of pigment in the eye and that in the Malpighian tubes.

Pigmentation in Malpighian tube transplants

In tests of Malpighian tubes for v^+ and cn^+ substances it was noted that in no case did the pigmentation of the tube implants appear to be modified by the host. In a further attempt to find out something as to the nature of pigment development in Malpighian tubes, transplants were made of tubes from all eye color mutants that differ from wild type in tube color, to wild type hosts. All operations were made in the late larval stage. The results are summarized in table 7. Tube color is apparently determined by the genetic constitution of the cells of the Malpighian tubes in all these cases. These results, considered in connection with experiments to be presented below, in which eye pigment and tube pigment extracts were injected, indicate that the yellow pigment normally present in larval tubes of certain genetic types is not taken up as such from the blood.

Do Malpighian tubes produce diffusible substances?

Since v^+ and cn^+ substances can be extracted from wild type Malpighian tubes in the late larval stage, a natural question is: are these substances produced by the cells of the Malpighian tubes, or do the tubes merely accumulate these substances from the blood? Various experiments have been made in an attempt to answer this and related questions.

Stages during which substances can be extracted from Malpighian tubes.—If it could be demonstrated that at a given stage Malpighian tubes contain no v^+ or cn^+ substance, it would be a simple matter to transplant the tubes at this stage and see whether they produce one or both substances subsequently. In an attempt to find such a stage of development, Ringer-extracts of wild type Malpighian tubes were made at various stages of larval development. The results of tests of such extracts are given in

TABLE 7

Pigmentation of Malpighian tubes of various genotypes, transplanted to wild type larval hosts three days after hatching. Under "number of individuals" female hosts, male hosts, and total number of individuals are given in the order listed. Two tubes (one pair) transplanted to each host, no distinction made between anterior and posterior pairs.

IMPLANT	COLOR OF LARVAL TUBES	SEX OF DONORS	NUMBER OF INDIVIDUALS			COLOR IMPLANTS AT ECLOSION
			♀	♂	Total	
bri	pale yellow	♀, ♂	9	5	14	pale yellow
bw	pale yellow	♀, ♂	8	4	12	pale yellow
ca	white	♀	8	6	14	white
car	pale yellow	♀	13	3	16	pale yellow
cm	pale yellow at base	♀, ♂	7	10	17	white
cn	pale yellow	♀, ♂	8	2	10	pale yellow
g^2	pale yellow at base	♀	5	5	10	white
lt	white	♀	9	3	12	white
ma	pale yellow	♀, ♂	10	5	15	pale yellow
p^p	white	♀	9	5	14	white
pr	pale yellow	♀, ♂	4	3	7	pale yellow
rb	pale yellow at base	♀, ♂	8	3	11	white
st	pale yellow	♀	7	2	9	pale yellow
v	pale yellow	♀	3	2	5	pale yellow
w	white	♀	6	5	11	white
cn bw	white	♀	8	4	12	white
v bw	white	♀	8	3	11	white

table 8. It is at once clear that both substances are present in the tubes at all stages of larval development tested; that is, at and following about twenty-four hours after hatching from the eggs. At this time the tubes are about one mm or less long and about 0.02 mm in diameter at their mid-regions. It would be rather difficult, but not impossible, to obtain extracts at earlier stages because of the small size of the tubes.

Accumulation of substances by Malpighian tubes.—In connection with the question of the production of substances by Malpighian tubes, it is important to know whether the tubes are capable of taking up v^+ and cn^+ substances from the blood if they are present. An obvious way to determine this is to add v^+ substance to the blood of vermilion larvae, the Malpighian tubes of which, of course, give negative results in tests for v^+ substance,

allow the larvae to live for a period of time and then make tests of the tubes. A similar test for the ability of cinnabar tubes to remove cn^+ substance from the blood is possible. In carrying out such experiments, rather concentrated Ringer-extracts of wild type Malpighian tubes were made. These extracts were injected into vermilion or cinnabar larvae (in some experiments vermilion brown and cinnabar brown larvae were used) at from four to twelve hours before puparium formation. Several hours after this injection the Malpighian tubes were removed from these original recipients and transplanted to vermilion brown or cinnabar brown larvae.

TABLE 8

Tests of Ringer extracts of wild type Malpighian tubes at different stages of larval development.

AGE DONORS (HRS. AFTER HATCHING)	NUMBER SETS OF TUBES EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR RECIPIENTS, MEAN AND RANGE
72±	40	?	40	*v bw*	10	7	17	3.1 (2.0–3.7)
72±	(same extract as above)			*cn bw*	7	10	17	3.2 (3.0–3.7)
48±	50	0.015	30	*v bw*	6	4	10	1.1 (0.7–1.7)
48±	(same extract as above)			*cn bw*	8	8	16	1.0 (0.7–1.3)
24±	60	0.005	6	*v bw*	1	1	2	3.3 (3.3)
24±	(same extract as above)			*cn bw*	4	0	4	2.6 (1.3–3.3)

(The experiments involving scarlet will be considered later). To avoid the possibility of transferring substances in solution in the blood, the tubes were rinsed twice in Ringer's solution. To be quite certain that substance in solution was not being transferred, Ringer's solution used in the first rinsing was tested. These rinsing-controls were negative (9 individuals in tests for v^+ substance and 8 in tests for cn^+ substance). The results of these experiments are summarized in table 9. It is quite clear that vermilion (or vermilion brown) tubes are capable of removing v^+ substance from the blood and that cinnabar (or cinnabar brown) tubes similarly remove cn^+ substance from the blood. Such experiments of course do not prove that v^+ and cn^+ substances normally found in the tubes is taken up from the blood.

In connection with experiments such as those described above, a note should be added regarding the effect of the extracts which contain the yellow pigment of the tubes on the color of the tubes of the original recipients. A very small amount of this yellow pigment does seem to be taken up, but it does not persist in the cells until eclosion. Thus, if a strong Malpighian tube extract is injected into vermilion brown larvae, which have

white tubes, the larvae appear quite yellow. If dissected after four or five hours, the Malpighian tubes are white or faintly yellow. At maturity, however, the tubes of such recipients are white; there appears to be no permanent change in color. This result agrees with the view, previously

TABLE 9

Measurements of the accumulation by Malpighian tubes of v^+ substance and cn^+ substance from larval blood. See text for details.

NUMBER EXPERIMENT	SOURCE OF EXTRACT	NUMBER SETS TUBES EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR RECIPIENTS MEAN AND RANGE
1953	wild	75	0.035	33	*v bw*	3	8	11	3.1 (2.3–3.3)
1954	wild (same extract as above)				*cn bw*	4	1	5	3.0 (2.7–3.3)
	(*v bw* Malpighian tubes taken from larvae of exp. 1953, 5½ hours after injection; transplanted to *v bw* larvae)					8	1	9	0.6 (0.3–1.3)
	(*cn bw* Malpighian tubes taken from larvae of exp. 1954, 6 hours after injection; transplanted to *cn bw* larvae)					3	0	3	0.0
1967	wild	50	0.02	27	*v*	6	4	10	Intermediate
	(*v* Malpighian tubes taken from larvae of exp. 1967, 2½ hours after injection; transplanted to *v bw* larvae)					10	3	13	0.9 (0.7–1.3)
1976	wild	80	0.02	22	*cn bw*	4	5	9	4.4 (4.3–5.0)
	(*cn bw* Malpighian tubes taken from larvae of exp. 1976, 2¾ hours after injection; transplanted to *cn bw* larvae)					7	5	12	0.5 (0.1–1.0)
1989	wild	50	0.02	21	*cn*	4	3	7	Intermediate
	(*cn* Malpighian tubes taken from larvae of exp. 1989, 2¾ hours after injection; transplanted to *cn bw* larvae)					3	10	13	1.0 (0.4–1.3)
2009	wild	50	0.015	15	*st*	.....			.....
	(*st* Malpighian tubes taken from larvae of exp. 2009, 4 hours after injection; transplanted to *v bw* larvae)					6	6	12	0.0
2023	wild	50	0.01	11	*st*	.....			.....
	(*st* Malpighian tubes taken from larvae of exp. 2023, 2¾ hours after injection; transplanted to *v bw* larvae)					6	7	13	0.0

expressed, that the yellow pigment normally found in wild type larval tubes is not taken up as such from the blood.

Are v^+ and cn^+ substances normally present in larval blood?—Knowing that Malpighian tubes can take up the two diffusible substances from the blood, it becomes important to know whether these substances are normally present in larval blood. The work of EPHRUSSI, CLANCY and BEADLE (1936), and BEADLE, CLANCY and EPHRUSSI (1937) indicates that v^+ sub-

stance is not present in the blood of wild type larvae. A similar result was obtained in tests of larval body fluids for cn^+ substance (HARNLY and EPHRUSSI 1937). It has been shown, however, that both of these diffusible substances can be extracted from wild type larval Malpighian tubes. Since it has been shown by THIMANN and BEADLE (1937) that these substances are inactivated when pupae are crushed in air, presumably by enzymic oxidation, the question is raised as to whether the negative tests obtained in direct transfers of larval fluid are the result of absence of the active substance in the blood or merely of its inactivation in air. Extracts of whole larvae are of course useless for answering this question since it is known that larval Malpighian tubes contain the active substances. Direct transfers of larval fluid are difficult to make without exposing large surfaces of the fluid to air. Additional tests have been made for both substances, but they have invariably given negative results. Direct transfers of fluid removed in a nitrogen chamber gave negative results for v^+ substance, but the tests were unsatisfactory because of technical difficulties. Larval fluid obtained by crushing larvae under nitrogen and centrifuging through an asbestos and glass wool filter have given positive results (in *v bw*, 6 individuals gave a mean value of 1.1; in *cn bw*, 13 tests gave a mean value of 1.4), but the active substances may have all come from the Malpighian tubes.

With the knowledge that vermilion and cinnabar Malpighian tubes are able to take up v^+ and cn^+ substances when these are added to the blood, it was possible to devise an experiment that would answer the question under consideration. Malpighian tubes from vermilion larvae were transplanted to wild type larvae about twenty-four hours prior to puparium formation. Shortly before puparium formation, the vermilion tube implants (dorsal or ventral pairs) were removed and re-implanted in vermilion brown hosts. In two experiments of this type, made at different times, the following results were obtained:

HOST	IMPLANT RECOVERED	NUMBER OF INDIVIDUALS	EYE COLOR OF HOSTS, MEAN AND RANGE
v bw	2 tubes	2, 0; 2	1.5 (1.0–2.0)
	1–1½ tubes	2, 5; 7	0.6 (0.1–1.3)
v bw	2 tubes	3, 2; 5	0.6 (0.1–1.3)

Similar results were obtained when the vermilion tubes were removed from the original hosts shortly after puparium formation.

It is evident, since vermilion tubes invariably give negative tests when transplanted directly to vermilion brown larvae (table 5), that the tubes grown for about twenty-four hours in wild type larvae had removed v^+ substance from the blood. It does not necessarily follow, of course, that in

taking up v^+ substance from larval blood, Malpighian tubes play an entirely passive role. By the nature of the test for it, it is obvious that v^+ substance may actually consist of two or more developmentally related substances. It is entirely conceivable that Malpighian tubes may take up one or more substances and release one or more different substances concerned in the test-reactions. If, however, v^+ substance is defined as anything that will produce the characteristic change in a test animal of appropriate genotype—and this is the only definition that we can at present make use of—it does follow that the negative results obtained in previous tests of larval fluid were the result of inactivation or, less probably, were obtained because the concentrations present were too low to give measurable tests.

Similar tests for the presence of cn^+ substance in larval blood are of course possible but they have not been made.

Attempts to exhaust Malpighian tubes of diffusible substances.—Various methods have been used in an attempt to exhaust living Malpighian tubes of v^+ and cn^+ substances with a view to seeing whether they could subsequently produce these substances.

Tubes of adult wild type flies, taken shortly after eclosion, transplanted to late larvae, give positive tests for both substances. In such tests, using females as donors, the following results were obtained:

HOST	NUMBER OF INDIVIDUALS	EYE COLOR OF HOSTS, MEAN AND RANGE
v bw	5, 7; 12	2.4 (2.0–3.7)
cn bw	5, 5; 10	0.5 (0.3–0.7)

In order to see whether the substances had been exhausted from adult tubes, extractions were made in hot Ringer's solution. From such extracts the following tests were obtained:

NUMBER SETS TUBES EXTRACTED	RINGER'S USED (cc)	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NO. OF INDIVIDUALS	EYE COLOR RECIPIENTS, MEAN AND RANGE
43	0.02	19	*v bw*	5, 1; 6	0.9 (0.3–1.3)
(same extract as above)			*cn bw*	5, 6; 11	1.0 (1.0–1.3)

Taken at face value, these results show that v^+ substance, if not cn^+ substance, is produced by the adult tubes after transplantion. In the injection tests an extract of about 8.5 single tubes per larva gave a test in *v bw* of 0.9, whereas two tubes (one pair) transplanted gave a test for v^+ substance of 2.4. In spite of this large difference in the activity of the transplanted tubes as compared with the extract, some doubt can be entertained as to the validity of the indicated conclusion; for one thing, such a conclusion is based on the assumption that a quantitative extraction was made.

On the assumption that the chances of exhausting Malpighian tubes for a given substance would be increased if the tubes were grown in a host deficient for the substance concerned, wild type tubes were transplanted to vermilion brown larvae and allowed to remain until eclosion of the hosts. The implants were then removed and tested in two ways, by extraction and by again transplanting to vermilion brown larvae. The extraction experiments gave results as follows:

NUMBER OF PAIRS TUBES EXTRACTED	RINGER'S USED (cc)	NUMBER LARVAE INJECTED	CONSTITUTION OF RECIPIENTS	NUMBER OF INDIVIDUALS	EYE COLOR RECIPIENTS MEAN AND RANGE
43	0.015	27	*v bw*	7, 4; 11	1.1 (0.7–1.7)
(same extract as above)			*cn bw*	6, 8; 14	0.9 (0.7–1.0)

Evidently these tubes contained small amounts of both substances. From tests made by transplanting to vermilion brown larvae, only two individuals were obtained. These gave a mean color value of 0.8 (0.7–1.0). Here there is no clear indication of production of v^+ substance after the second transplantation.

TABLE 10

Attempts to exhaust wild type Malpighian tubes of v^+ and cn^+ substances by extraction with Ringer's solution at 25°C. See text for details.

NUMBER SETS TUBES EXTRACTED	RINGER'S USED (cc)	TREATMENT	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR OF RECIPIENTS, MEAN AND RANGE
30	0.02	Extracted with cold Ringer's for 65 to 120 minutes	17	*v bw*	3	2	5	3.2 (3.0–3.3)
				cn bw	4	6	10	2.8 (2.0–3.3)
30	0.02	Soaked in cold Ringer's for 60 to 90 min., extracted with hot Ringer's	19	*v bw*	5	1	6	1.4 (0.7–2.0)
				cn bw	7	2	9	1.9 (1.3–2.3)
30	0.02	Soaked in cold Ringer's for 75 to 115 min., rinsed, extracted with hot Ringer's	22	*v bw*	4	6	10	2.1 (0.7–2.3)
				cn bw	8	1	9	2.1 (1.3–2.7)

It is known that both v^+ and cn^+ substances diffuse from wild type Malpighian tubes if these are allowed to stand in Ringer's solution at room temperature for a short time (BEADLE 1937a). A series of experiments was made in which tubes were partially extracted in Ringer's solution at 25°C and then extracted with hot Ringer's solution or transplanted to test lar-

vae. Tests of cold (25°C) Ringer-extracts of tubes from wild type larvae approaching puparium formation are summarized in table 10. Relatively strong tests are recorded for both substances. In order to find what proportion of the two substances were extracted by this treatment, experiments were made in which tubes were allowed to stand in Ringer's solution for time intervals of the order of one to two hours. The Ringer's solution was pipetted off, and hot Ringer-extracts were made as usual. Comparisons of the two types of extracts show that a good deal more than half of the diffusible substance had gone into the cold Ringer's solution in from one to two hours (table 10). The results of transplantation tests of tubes allowed to stand in Ringer's solution at 25°C for various intervals of time (table 11) show no convincing evidence of a decrease in their activity. For intervals longer than about 90 minutes, entire implants were not recovered at emergence; fragments only were recovered and in many cases nothing was found on dissection.

TABLE 11

Transplantation of wild type Malpighian tubes from late larvae after standing in Ringer's solution at 25°C. Comparable data grouped by horizontal spacing.

TIME IN RINGER'S SOLUTION (MIN.)	HOST	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
		♀	♂	Total	
0	*cn bw*	3	1	4	2.8 (1.7–3.0)
35	*cn bw*	3	2	5	2.8 (2.7–3.0)
55	*cn bw*	3	1	4	2.0 (1.0–3.0)
90	*cn bw*	0	1	1	3.0
0	*cn bw*	10	3	13	3.0 (2.3–4.0)
145–150	*cn bw*	7	9	16*	2.6 (2.0–3.3)
65–110	*v bw*	2	6	8*	2.5 (1.7–3.7)
90–120	*v bw*	3	0	3*	1.8 (1.3–2.0)

* Intact implants not recovered; generally small pieces or nothing found at eclosion.

From the numerical values obtained in the transplantation and extraction tests just considered, one might conclude that tubes partially extracted with cold Ringer's solution produce additional substances after transplantation. On the strength of these experiments alone, however, the evidence cannot be considered entirely convincing.

Production of cn^+ substance by vermilion Malpighian tubes.—According to the interpretation previously given of the relation between v^+ substance and cn^+ substance vermilion flies should be deficient in cn^+ substance.

Table 6 shows that vermilion Malpighian tubes transplanted to cinnabar brown larvae, modify the eye color slightly indicating production or release of cn^+ substance. This can be interpreted in the same way as we have previously interpreted the formation of cn^+ substance by vermilion eye-implants, that is, production of cn^+ substance by vermilion Malpighian tubes when grown in the presence of v^+ substance. If a vermilion fly is totally deficient in both substances, as opposed to having sub-threshold concentrations, and if the above interpretation is correct, extracts of vermilion Malpighian tubes should contain no cn^+ substance. Actually it is found that such extracts are negative in tests for cn^+ substance (table 12). We can now invert the argument in the following way: Extracts of

TABLE 12

Tests of concentrated extracts of Malpighian tubes of vermilion and cinnabar larvae.

DONORS	NUMBER SETS TUBES EXTRACTED	RINGER'S USED (cc)	NUMBER LARVAE INJECTED	CONCENTRATION RATIO	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE-COLOR RECIPIENTS, MEAN AND RANGE
v	36	?	19	1.9	*v bw*	2	2	4	0.0
v	(Same extract as above)			1.9	*cn bw*	10	5	15	0.0
v	32	0.01	11	2.9	*v bw*	5	6	11	0.0
v	45	0.01	13	3.5	*cn bw*	8	4	12	0.0
v	100	0.012	11	9.1	*v bw*	5	1	6	0.0 (?)*
v	(Same extract as above)			9.1	*cn bw*	5	0	5	0.0
v	235	0.03	21	11.2	*v bw*	10	1	11	0.0
v	80	0.01	8	10.0	*cn bw*	1	2	3	0.0†
cn	60	0.01	16	3.8	*v bw*	0	3	3	1.1 (0.3–2.0)
cn	(Same extract as above)			3.8	*cn bw*	7	3	10	0.0
cn	80	0.01	9	8.9	*v bw*	1	0	1	3.0
cn	(Same extract as above)			8.9	*cn bw*	4	1	5	0.0

* Only three comparable controls available for females; male clearly negative.

† Injections not entirely satisfactory; some fluid lost.

larval vermilion tubes contain no cn^+ substance, at least not in measurable quantities. Such tubes, transplanted to cinnabar brown larvae, modify the eye color, showing that cn^+ substance has been supplied to the eyes by the Malpighian tubes. It follows, therefore, that vermilion Malpighian tubes, in the presence of v^+ substance supplied by the cinnabar brown host, have produced cn^+ substance.

The evidence considered above indicates that Malpighian tubes do produce v^+ and cn^+ substances, at least under certain conditions. Whether they also take up appreciable amounts of these substances from the blood in the larval stage is another question that cannot be answered at present. If they do, there presumably must be an undiscovered source of these sub-

stances in the larvae for there is evidence that fat bodies produce v^+ substance only after puparium formation and it seems improbable that eye tissue can produce appreciable amounts of the two substances in larval stages. The question must remain open until additional experimental evidence is at hand.

POSSIBILITY OF SUB-THRESHOLD CONCENTRATIONS OF DIFFUSIBLE SUBSTANCES

In earlier papers on diffusible substances in relation to eye color development, the tacit assumption was made that a vermilion fly has no v^+ or cn^+ substance (BEADLE and EPHRUSSI 1936). Later it was pointed out (EPHRUSSI and BEADLE 1937a; BEADLE and EPHRUSSI 1937) that such an assumption is not justified. Extraction experiments provide a means of answering this question experimentally. If a vermilion fly differs from a wild type fly merely in having less v^+ and cn^+ substances, it should be possible to demonstrate the presence of these substances by using concentrated extracts. A similar test should be possible in determining whether a cinnabar fly contains any cn^+ substance. Such tests have been made by extracting large numbers of Malpighian tubes in small volumes of Ringer's solution; the results are summarized in table 12. Extracts of vermilion tubes give negative results in tests for both substances up to concentration-ratios of 11.2 (ratio of number of sets of tubes extracted to volume of extract injected per larva). Extracts of cinnabar tubes give positive tests for v^+ substance, but negative tests for cn^+ substance. The highest concentration-ratio investigated was 8.9. By means of such experiments, it is of course impossible to prove that the substance in question is absent; the concentration can always be assumed to be below the test threshold. For practical purposes, however, these experiments can be taken to favor our original assumption, that is, absence of both substances in vermilion and absence of cn^+ substance only in cinnabar.

TESTS FOR SUBSTANCES IN OTHER ORGANS

It is known that under certain conditions eye tissue is capable of producing and liberating v^+ and cn^+ substances (EPHRUSSI and BEADLE 1937b). Extracts of eyes (whole heads) of freshly emerged mature wild type flies made up in hot Ringer's solution give weak positive tests for both v^+ and cn^+ substances (table 13). Apparently somewhat more of both of these substances is available than is normally used by the eyes. This result is in agreement with the tests of extracts of Malpighian tubes of adult flies in showing the presence at this stage of small amounts of both substances. HARNLY and EPHRUSSI (1937), in experiments in which body fluid was injected, found traces of both substances in pupae just before and

at the time of eclosion. Extracts of scarlet eyes give results similar to those for wild type (table 3). These will be discussed in a later section.

TABLE 13

Tests of Ringer-extracts of adult eyes (heads) for v^+ and cn^+ substances.

DONORS	NUMBER HEADS EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR RECIPIENTS MEAN AND RANGE
wild	70	0.04	40	*v bw*	9	8	17	0.4 (0.2–0.8)
	(same extract as above)			*cn bw*	6	8	14	0.3 (0.2–0.4)
st	60	0.03	22	*v bw*	6	3	9	0.5 (0.3–0.6)
	(same extract as above)			*cn bw*	2	7	9	0.2 (0.1–0.3)

Extracts of adult eyes contain eye-pigment. Shortly after injection of such extracts, the Malpighian tubes of the recipients become bright reddish-orange in color. This pigment, taken up from the blood, largely disappears in the adult, but traces of it may remain. MORGAN (1930) has studied the uptake of eye-pigment by the Malpighian tubes following injury to the eye, feeding larvae crushed eyes, and following injection of eye-pigment into adult flies. The pigment accumulated by the tubes following injection of eye-pigment appears to be quite different from that normally found in the tube; it is quite red as compared with the yellow pigment characteristic of wild type Malpighian tubes.

Extracts of testes of adult wild type flies made up in hot Ringer's solution are very similar in color to extracts of Malpighian tubes. Tested for v^+ and cn^+ substance, the following results were obtained:

NO. PAIRS TESTES EXTRACTED	RINGER'S USED (CC)	NO. LARVAE INJECTED	CONSTITUTION RECIPIENTS	NO. OF INDIV.	EYE COLOR RECIPIENTS
55	0.02	24	*v bw*	8, 4; 12	0.0
	(same extract as above)		*cn bw*	3, 7; 10	0.0

Extracts of wild type *D. virilis* testes which are orange-red in color gave negative results in tests for the two substances made by injection into larvae of *D. melanogaster* (13 individuals in v^+ substance test, 10 in test for cn^+ substance).

THE SCARLET CHARACTER

Although scarlet flies are known to produce v^+ substance (BEADLE and EPHRUSSI 1936; see also tests of fat bodies, this paper), Malpighian tubes of scarlet larvae transplanted to vermilion brown have no effect on the eye color of the host. On the assumption that such tubes might take up

v^+ substance from the blood after its production by the fat bodies, tests were made by transplanting scarlet tubes at various stages of development. The results were negative at all stages of pupal development up to and including the adult stage (table 14). Evidently scarlet tubes do not

TABLE 14

Tests of scarlet Malpighian tubes taken at various stages of development after puparium formation and transplanted to vermilion brown larvae. Unless otherwise indicated, one pair tubes, anterior or posterior, transplanted in each test.

TIME AFTER PUPARIUM FORMATION (HRS.)	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS
	♀	♂	Total	
0– 2.5	6	5	11	0.0
20 –21.5	6	0	6	0.0
47.5–55.8	9	6	15	0.0
66.2–74.8	8	6	14	0.0
66.8–75.5 (4 tubes)	3	4	7	0.0
Just before eclosion	3	4	7	0.0
Just after eclosion	8	2	10	0.0

take up v^+ substance from the blood. A further test of this was made by injecting concentrated extracts of wild type tubes into scarlet larvae some hours before puparium formation. Two to $4\frac{1}{2}$ hours later the tubes were removed from these scarlet larvae and transplanted to vermilion brown test-larvae. Again the results were negative (table 9), indicating that under these conditions scarlet tubes do not take up detectable quantities of v^+ substance. Tests were then made to see if scarlet tubes in some way inactivate v^+ substance. Fifty sets of wild type Malpighian tubes were extracted in 0.02 cc of Ringer's solution in the usual way. This extract was divided into two samples of equal volume. One sample was kept as a control. To the other, 25 sets of scarlet tubes were added (without heating), allowed to stand at 25°C for $2\frac{1}{4}$ hours, and then heated and centrifuged. Both extracts were then tested by injection into vermilion brown larvae. The results of these tests follow:

EXTRACT	NO. LARVAE INJECTED	NO. OF INDIVIDUALS	EYE COLOR RECIPIENTS, MEAN AND RANGE
st tubes added	9	3, 6; 9	4.2 (4.0–4.3)
control	12	5, 6; 11	4.1 (4.0–4.3)

Under the conditions of the experiment there was evidently little or no inactivation by the scarlet tubes.

Extracts of the eyes of scarlet adult flies show that v^+ substance is present (table 13).

The facts given above provide an interesting clue as to the possible nature of the scarlet character. Scarlet belongs to the vermilion-like group of eye colors (SCHULTZ, 1935); phenotypically it is indistinguishable from vermilion and cinnabar. WRIGHT (1932) has pointed out that a simple interpretation, in terms of physiological development, of the fact that the double recessive vermilion scarlet is not distinguishable from the single eye colors and of the similar interactions of vermilion and scarlet with brown (both double recessives with brown are practically white) follows the assumption that the wild type alleles of both vermilion and brown are concerned with different steps in a chain reaction necessary for the development of wild type eye color. We have evidence from transplantation experiments that the formation of v^+ substance may be regarded as one of the steps in such a chain reaction, the production of cn^+ substance another. In scarlet both substances are present and we can suppose that the break in the series of chain reactions is in the utilization of one or both of these substances in the eye. On this interpretation, the scarlet character, so far as the end result is concerned, is the same as vermilion or cinnabar; the difference lies in the manner in which the end result is brought about. The inability of scarlet Malpighian tubes to take up v^+ substance from the blood lends some support to this interpretation in that it shows that a mechanism similar to that required by the suggested hypothesis must exist in the cells of the Malpighian tubes.

SUPPRESSOR OF VERMILION

BEADLE and EPHRUSSI (1936b) concluded from various transplantation experiments that the recessive gene suppressor of vermilion (*su-v*), when combined with vermilion, somehow partially restores the reaction leading to the formation of v^+ substance. Tests of *su-v v* fat bodies for v^+ substance are negative (table 1) as are tests of pairs of Malpighian tubes of the same constitution (table 5). Four Malpighian tubes of a *su-v v* fly give a positive test for v^+ substance, but so weak that, by itself, the test would scarcely be convincing. To test the results previously obtained, two series of eye-transplants involving suppressor of vermilion were made. The results follow:

IMPLANT	HOST	NO. OF INDIVIDUALS	PHENOTYPE OF HOST	PHENOTYPE OF IMPLANT
su-v v	*v bw*	5, 5, 11, 0; 21	*v bw*	*su-v v*
v bw	*su-v v*	10, 0, 8, 2; 20	*su-v v*	Intermediate between *v bw* and *bw*

The first shows that a *su-v v* eye-implant does not release any appreciable quantity of v^+ substance; presumably the implant itself produces v^+ substance, but not enough to give completely wild type eye color. The second result shows that a *su-v v* fly produces some v^+ substance, but not enough

to bring about a complete change from vermilion brown to brown. This confirms the results reported in our earlier paper.

OCELLUS COLOR

Both vermilion brown and cinnabar brown flies have white ocelli. It was found (BEADLE 1937a) that when a strong modification of the eye color of a vermilion brown fly is brought about by supplying v^+ substance, the ocellus color is modified toward brown. The corresponding change in ocellus color in cinnabar brown flies was not obtained. The experiments reported here confirm this finding. Aside from noting these relations, no experiments have been especially directed toward a more thorough study of ocellus color.

DISCUSSION

The relation of the studies reported here to the results obtained by STURTEVANT (1932) in his investigations of mosaic individuals of *D. simulans* made up of vermilion and not-vermilion tissue are somewhat puzzling. STURTEVANT found a strong correlation between the type of pigment developed in vermilion eye-tissue and the constitution of the gonads. Where both gonads were not-vermilion and female, genetically vermilion eye tissue developed not-vermilion pigmentation. On the other hand, when the gonads were vermilion and male, vermilion eye-tissue developed the characteristic vermilion color. There were a few exceptions to this behavior. In previous transplantation work (EPHRUSSI and BEADLE 1935), we were unable to find any evidence of a direct relation between gonads and v^+ substance. In the studies reported above, the experiments involving ovaries give no indication that presence or absence of wild type ovary implants has any influence on the end result. The only interpretation that the writer can offer is that the relations found by STURTEVANT result from a relation in terms of cell lineage between gonads and the organs which produce v^+ substance. This would mean that in elimination gynandromorphs with female gonads, such as were studied by STURTEVANT, the probability would be high that a considerable portion of the tissue of the fat bodies and, presumably, also of the Malpighian tubes, would be female (not-vermilion). Correspondingly, in individuals with male gonads, the probability would be high that fat bodies and Malpighian tubes would be male (vermilion). On such an interpretation relation between gonads and eye-pigment differentiation in gynandromorphs would, of course, be indirect; this would agree with the results of transplantation studies. There appears to be little point in discussing the facts regarding embryological development in relation to the interpretation suggested above; the available knowledge of the cell-lineage relations of the organs concerned is insufficient.

SUMMARY

1. In general, the experiments reported were designed to determine where, when, and how the diffusible substances concerned in eye color development are produced.

2. Fat bodies of all eye color mutants studied, except those from vermilion larvae, are shown to produce v^+ substance following transplantation to appropriate test animals.

3. Extracts of fat bodies gave negative results in tests for v^+ substance; presumably they produce v^+ substance only after puparium formation.

4. Fat bodies do not produce cn^+ substance.

5. Malpighian tubes of wild type larvae release both v^+ and cn^+ substances following transplantation. Both substances can be extracted from the larval tubes with hot Ringer's solution.

6. The white distal regions of the anterior pair of Malpighian tubes were negative in all tests for the two substances.

7. Malpighian tubes from larvae differing in genetic constitution with respect to eye color genes may vary in the amount of v^+ and cn^+ substance released. There is a general, but not simple, relation between release of diffusible substances and depth of pigmentation of the Malpighian tubes.

8. Malpighian tube color is autonomous in all combinations studied when tubes are transplanted in late larval stages.

9. Both v^+ and cn^+ substances are present in the Malpighian tubes of wild type larvae at and following the earliest stage studied—about twenty-four hours after hatching from the eggs.

10. Larval Malpighian tubes normally not containing or producing a particular diffusible substance, can take up that substance if it is added to the larval blood; vermilion tubes can take up v^+ substance and cinnabar tubes can take up cn^+ substance.

11. By making use of the fact just mentioned it has been possible to demonstrate the presence of v^+ substance in the blood of wild type larvae during some part or all of a 24-hour interval just prior to puparium formation.

12. Various attempts were made to exhaust living Malpighian tubes of their diffusible substances; these attempts were partially successful.

13. The evidence favors the view that Malpighian tubes produce at least a part of the substances that can be obtained from them.

14. Concentration experiments failed to demonstrate the presence of either v^+ or cn^+ substance in vermilion Malpighian tubes or the presence of cn^+ substance in cinnabar tubes.

15. It is suggested that there is a block to the utilization of v^+ or cn^+ substance, or both, in scarlet flies.

16. Under the conditions of the experiments reported, ocellus color is autonomous in cinnabar, dependent in vermilion.

17. The results of the work on fat bodies and Malpighian tubes are discussed in relation to STURTEVANT'S studies of gynandromorphs in *D. simulans* in which vermilion eye color was concerned.

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THE STRUCTURE AND THE DEVELOPMENT OF THE SALIVARY GLAND CHROMOSOMES OF SIMULIUM

THEOPHILUS S. PAINTER AND ALLEN B. GRIFFEN
University of Texas, Austin, Texas

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INTRODUCTION

THE discovery that individual genes are associated with definite chromatic "bands" along the giant salivary gland chromosomes of *Drosophila melanogaster* (PAINTER 1934) focussed our attention on the nature of these bands and on the structure of these chromosomes in general. At the present time two very different interpretations are given to the observed phenomena. On the one hand we have the concept, suggested independently by BRIDGES (1935) and by KOLTZOFF (1934), that these giant chromosomes represent prophase elements which have undergone a number of divisions. In effect each chromosome is a bundle of chromonemata; the "bands" are rows or discs of homologous chromomeres, the number of which will depend on how many times the original chromonema is split; and the achromatic spaces between the bands contain the threads which connect the chromomeres of the separate chromonemata. This view has been widely accepted, alike by cytologists and geneticists, and a considerable amount of work has appeared supporting it, the most outstanding being the studies of BAUER (1935, 1936) dealing with the chromosomes of Chironomus larvae. On the other hand, METZ (and his students) does not believe that the original prophase chromosome has divided a number of times; instead, he thinks that the giant chromosomes have a honeycomb structure and are made up of little droplets of material. When the chromosome is stretched these droplets elongate forming "alveoli"; and the side walls of these alveoli, where they touch, have been mistakenly interpreted by others as chromonema threads. Between the abutting ends of alveoli, as a sort of chinking, chromatin is deposited forming a continuous disc or band across the chromosome. Thus the threads and chromomeres of the chromonema interpretation are regarded, by METZ, as different aspects of a system of discontinuous alveoli (in a longitudinal direction) and continuous discs or plates of chromatin.

Needless to say, the admitted correlation between certain visible elements and gene loci makes it highly desirable that we understand the physical structure of the giant chromosomes as completely as possible. Both of the interpretations referred to above are based on essentially similar observations on fully differentiated salivary chromosomes, and while more study of the facts is necessary, a moment's consideration will

indicate that fully as important for an understanding of the adult structure is the ontogenetic history of the elements. This was recognized early in the development of the salivary chromosome work, at this laboratory, but unfortunately *D. melanogaster* has not proved favorable for this type of study. Some eighteen months ago we began working on Simulium, principally *S. virgatum*, the larvae of which are common about Austin most of the year. In this form the chromosomes are about three times as broad as in the fruit fly; and what is far more important, somatic synapsis does not lead to such an intimate association of the component parts, so that one can generally distinguish individual chromomeres and the connecting strands, and from a study of early stages determine how the various type of bands are formed.

MATERIAL AND METHODS

Larvae collected in the streams about Austin can be held alive in the laboratory until they pupate, provided they are kept in a cool room in a stream of fast flowing water.

For a study of living nuclei larvae were placed on a clean slide, carefully dried and covered with paraffin oil; in this medium the salivary glands were dissected out and, surrounded by a drop of body fluid, were mounted under a coverslip. At no time were the glands exposed to the air or to salt solutions. If the effects of fixatives were to be studied the coverslip was sealed except for a narrow space at each end which allowed us to draw new fluids under the cover with filter paper.

A number of different fixation methods and stains were tried but none proved more useful than either temporary or permanent aceto-carmine preparations. Wratten filters 61 and 62 are indispensable for the study of the latter with artificial light, and oblique illumination brings out many fine details not ordinarily seen.

A few observers have described and given photomicrographs of living salivary chromosomes showing that they have essentially the same form before and after fixation with aceto-carmine. DOYLE and METZ (1935), however, report that the unaltered living chromosomes from active larvae of *Sciara* appear as optically homogeneous cylinders within the nucleus and show no sign of the bands until after fixation. They also state that after fixation with aceto-carmine there is a shrinkage in the chromosome diameter of from 50 to 70 percent. Such a serious indictment of the method so extensively used for staining requires a careful checking.

In Simulium the unaltered living chromosomes appear very much as they do in a well fixed and stained aceto-carmine preparation. The various types of bands are visible; chromomeric vesicles, both large and small, are clearly apparent and in the spread-out region the longitudinal fibers

are visible. We have carefully measured the diameters of chromosomes before and immediately after fixation, on photomicrographs, and find a shrinkage of chromosome diameter of from 10 to 17 percent. Fixation alters the image of the chromosome somewhat in that some of the chromomeric vesicles appear to swell a little but on the whole the images before and after preservation are remarkably similar. This indicates that in Simulium we may accept the validity of the fixation image as far as major details are concerned.

In somatic equatorial plates in Simulium there are six chromosomes with medial or sub-medial spindle fiber attachments; in the salivary gland nuclei we find three pairs of more or less intimately synapsed elements. There is no chromocenter in this form, and somatic synapsis[1] appears to be more intimate than in the European form studied by GEITLER (1935). For the most part the salivary chromosomes exhibit throughout their lengths the typical banded form, and there is the strictest sort of regimentation of the chromomeres. We shall call this type of area "euchromatic." However, in the longest chromosome (pair) sub-medially there is a region where homologues do not unite in intimate synapsis, and even within one homologue, or one chromatid, the chromomeres do not form compact bands. This is accompanied by a spreading of the chromosome constituents which makes this region favorable for the study of certain details. For the present we call this the "spread-out" region, although it may be related to the heterochromatic areas of other species of Diptera.

The twisting of homologous chromosomes about each other, which accompanies somatic synapsis, is easily seen in Simulium and in euchromatic areas amounts to about one full turn to each four diameters of the synapsed chromosomes. In the spread-out region the twisting is less pronounced; in younger ontogenetic stages the mates often lie parallel for a relatively long distance, and we observe that each homologue is split (fig. 4). We have then, in reality, within each giant chromosome four chromosomes or chromatids and as one focusses carefully on any band four parts are generally apparent. Similarly, in the achromatic spaces there are four bundles of "fibers" running at somewhat different angles. It is highly important that this tetrapartite nature of the giant chromosome be kept in mind because when the chromosome is greatly flattened by coverglass pressure, a single band may be separated into four sections and the fibers of each chromatid, running at different angles, may give the misleading impression of a net-work.

[1] Some authors use the term "somatic synapsis" to include not only the close apposition of homologous chromomeres, the sense in which Painter uses this term, but also the more general phenomenon long known as "somatic pairing." It may well be that both phenomena are basically due to the same force or forces, but their objective expressions are so different that it seems wise to use both terms in their original senses.

THE STRUCTURE OF THE EUCHROMATIC AREA

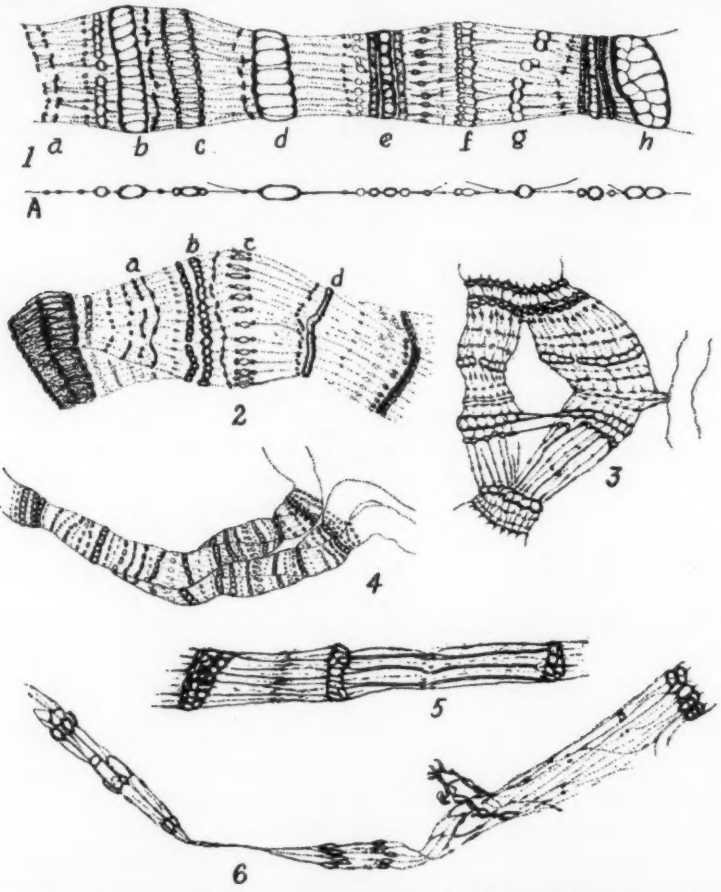
As one examines a series of well preserved and stained aceto-carmine preparations he finds three different expressions of the same chromosome organization. The first, which we shall call the "large vesicle" type, is shown in figure 1. The enlarged chromomeric vesicles can be readily observed in living nuclei and hence this form is assuredly "normal" in the sense that it appears in the unaltered living chromosome. The "small vesicle" form is shown in figure 2 and differs essentially from the first only in that the vesicles are small and, as a consequence, the chromomeres appear more clearly as separate entities. We regard this second expression as "normal" because in many living nuclei enlarged vesicles do not appear and yet the bands are double. In some preparations the chromomeric vesicles are scarcely visible as separated spaces; in such chromosomes the chromatic hulls remain intact, however, and we have coarse granular bands or lines running transversely across and through the element. Whether or not this form is "normal" we are unable to say because such details can not be made out in living chromosomes. METZ and GAY (1935) claim that in Sciara the character of the image given by a band is greatly affected by the nature of the dissecting medium; but since according to DOYLE and METZ the unaltered living chromosome is optically structureless, the basis for this conclusion is not clear. In Simulium we have used throughout this study the same methods of handling, fixing and staining the glands; and since in any given larva one expression of form dominates, we are inclined to think that the source of variation is extrinsic to the technique.

Figure 1 is a camera lucida drawing made from a permanent aceto-carmine mount. So far as possible we have drawn in only those details which show on the surface of the upper half of the chromosome. But since the chromomeres form a sort of plate or disc transversely through the chromosome, as one focusses downward superficial units disappear and deeper lying ones come into view and we may have included a few of the deeper lying features in the surface view. Likewise, while it is clear enough that the longitudinal fibers connect with the chromomeres of the compact bands, such as at *d*, we cannot be sure that the fiber drawn goes to the exact chromomere illustrated in the figure.

Figure 1A is a semi-diagrammatic representation of the types of chromomeres observed in figures 1 and 2, and of the ways in which these are connected.

Beginning at *a* there are two rows of dot-like chromomeres which tend to associate in pairs. Individually these are about the same size and stain deeply with no visible cavity or space inside the chromomere; there are about 16 in each row, across the surface of the chromosome. Fine lines arise

from each of these chromomeres and pass to the next row on the right which contains 15 or 16 elements. The chromomeres of the third row are much larger and there is a rim or hull of chromatin surrounding a non-staining center which we call the vesicle. The chromatin in the vesicular



FIGURES 1–6. Euchromatic region. Figure 1. A camera lucida drawing showing the details which appear on the upper surface of a fully developed salivary chromosome of the large vesicle type. Figure 1A is a semi-diagrammatic sketch of the types of chromomeres which make up the bands of figure 1. Figure 2. A chromosome of the small vesicle type showing the same region as figure 1 *a* to *e*. Figure 3 was made from a chromosome after treatment with KOH to remove part of the chromatin. Figure 4, drawn at a lower magnification, shows that each homologue is split prior to somatic synapsis. Figure 5 shows how unusually heavy fibers are formed when the chromomeres of a band are displaced. Figure 6 shows single and compound fibers in a young chromosome in about the 8-strand stage.

wall tends to lie on the sides next to the fibers giving a clam-shell effect, to use BRIDGES' figure of speech (1935).

The band labelled *b* is made up of 15 or 16 large vesiculate chromomeres closely pressed together. The chromatin is largely confined to the free surfaces or ends of the chromomeres, where it forms two deeply staining saw-tooth lines across the chromosome. The resulting double band effect is what is so commonly observed in all types of salivary chromosomes. Between the double band the adjoining walls of the chromomeric vesicles appear as fine lines. Here it should be remarked that the localization of the chromatin may be, in part, the result of fixation; for while in the living chromosome many bands appear double, showing that in life there is asymmetry in the distribution of chromatin, it is also true that after fixation the bands appear to be somewhat broader, as though the acetic acid caused the vesicles to swell a little. The vesicles of band *b* are all about the same size but it is not uncommon to observe, even in living chromosomes, a band with one or two vesicles which are considerably larger than the rest. The explanation for this will be given later.

To the right of the band *b* there is a row of non-vesiculate chromomeres which are rather large for this type. These are connected to the chromomeres of adjoining rows by fine parallel threads.

The band at *c* merits careful study, for this type is very common. Superficially it appears as a "double" band; however, the edges are not sharply delimited, as at *b*, but are fuzzy. Close inspection shows that there are present three rows of vesiculate chromomeres which are closely joined in a linear direction. A similar type of band is shown at *f;* here at one end the chromomeres are not vesiculate.

The band at *d* is much like *b* except that the chromatic edges are thicker, stain more densely, there are fewer vesicles, and the chromomeres are larger. Instead of 15 or 18 units at the surface, there are about 8 at *d*. On close inspection, especially with oblique illumination, it will be observed that many fine lines arising from the chromomeres at *c* join in pairs before they connect with the dot-like chromomeres just to the left of *d*. While we realize that chromomere counts are open to error when we try to enumerate the number lying at the upper surface, it is evident that the number at *d* and a few more bands to the right is much less than at the left in the figure. Such differences in adjoining bands are not uncommon and ontogenetic stages indicate that the cause (or causes) lies within the chromomeres themselves. Some seem to undergo fission more readily than others.

At *e* there are five rows of chromomeres rather closely associated; in small or less perfectly preserved chromosomes this would be classed as a "broad and fuzzy" band. Between *e* and *f* the threads seem to bifurcate and in the latter band there are 17 or more chromomeres. At *g* the number

is lower again and once more we see the threads associating in pairs. The band at *h* is somewhat unusual for there are two rows of large vesiculate chromomeres closely pressed together, the chromatin appearing on the free edges.

Figure 2 is a camera lucida drawing taken from a chromosome of the small vesicle type, showing the same region as figure 1 *a* to *e*. One has no difficulty in recognizing corresponding bands, in the two figures; they are made up of the same kinds of chromomeres, in the same sequence and with about the same number of elements in the rows. In general, in figure 1 the chromomeres are larger than in figure 2, and in the case of bands *b* and especially *d* the difference is very great. The amount of hypertrophy which the chromomeres of a band undergo seems to depend on some intrinsic factor and not on its position. Thus the chromomeres in the row just to the left of *b*, are perhaps twice as large in figure 1 as in figure 2, while the difference in band *b* itself must be of the order of 4 or 5 to 1. Similarly, in the row just to the left of band *d*, the chromomeres are not greatly different in size, while in *d* the ratio must be 6 or 8 to 1. Going hand in hand with these size differences in the vesicles we note differences in the way the chromatin is laid down. In figure 2, in the band *b* the chromatin more or less surrounds each vesicle giving a scalloped effect, while in figure 1, the chromatin is confined to the free edges. At *d*, in figure 2, the chromatin in the right hand edge is much heavier than on the left, but in figure 1 there is no sensible difference between the two sides.

The granular form of chromosome is not shown, but it differs from figure 2 in two ways. First, the chromatin appears to be more localized at two sides of the chromomere, just as in band *g* of figure 1 and in the absence of visible vesicular walls we get a scalloped and coarsely granular double band across and through the chromosome. In the second place, we are usually unable to detect any longitudinal fibers in these granular chromosomes; at any rate, there are no prominent threads. This absence of discrete chromomeres and of connecting fibers in granular chromosomes seems to warrant the conclusion that such elements must be regarded as aberrant in type though this may be a normal stage in living chromosomes.

Figure 3 was taken from a slide in which the chromosomes were first preserved in 45 percent acetic acid then spread and treated for about an hour with caustic potash to remove some of the chromatin. The stain used was Delafield's haematoxylin followed by eosin.[2] In the area illustrated the

[2] Salivary glands are fixed for about 10 minutes in 45 percent acetic acid and then placed on a slide and crushed under a coverslip, just as in making an aceto-carmine mount. The coverslip is now removed and after rinsing in water the slide and cover are placed in a .1 percent solution of potassium hydroxide where they are left for 10 minutes to 1½ hours. After washing well, the tissue is stained in Delafield's haematoxylin followed by eosin and mounted in euparal from 95 percent alcohol.

coverglass pressure has separated the homologues for a space but the chromomeres of two bands have stuck together so that those on the edge are greatly drawn out. It should be remembered that when the nucleus was preserved it was intact and that the spreading came afterwards. We do not know, therefore, whether the cohesion between the chromomeres of a band obtains in life, but it is very strong in preserved material. In general, figure 3 gives us the same picture of chromosome organization as figure 1; but the removal of part of the chromatin enables us to see somewhat more clearly the individual chromomeres which go to make up the bands.

In this study we have constantly looked for evidence which would indicate that each of the four chromatids was encased in some sort of pellicle and that lines of tension in this covering produces part of the longitudinal "fibers." It is true that in greatly stretched areas, or areas where the rows of chromomeres are displaced, we find fibers which are not ordinarily seen; but the explanation for this is very simple and obvious, and at no time have we found any evidence for a separate covering.

In figure 1 the threads which appear to connect the chromomeres of adjoining rows are extremely fine and are not easily seen except in subdued light or better still, with oblique illumination. They do not stain appreciably with aceto-carmine, or any of the other stains employed, and their visibility seems to depend more on their refractive properties than on anything else. The fibers shown are only those which lie at the surface; but as one focusses through the chromosome, more come into view until the lower edge of the chromosome is reached. When the chromomeres are in regular rows the fibers are smooth in outline, are about the same thickness, as far as we can tell, and run parallel from one band to the next when the chromomeres of adjacent rows are equal in number. If one examines greatly stretched chromosomes, however, he will find areas where some threads are much thicker than others. We have spent a good deal of time in determining how these thick threads are formed. As it turns out the explanation is simple; stretching or pressing on the chromosomes often displaces the chromomeres of a row so that several of the fine connecting fibers are brought into close apposition, forming a thicker thread.

Figure 5 is a drawing of a portion of a chromosome which was greatly stretched when the nucleus was crushed by coverglass pressure. Note that the chromomeres in the band to the right are not arranged in a regular transverse row but have been crowded into an irregular heap. From this heap three prominent lines arise and pass on the left to another broken-up row of non-vesiculate chromomeres. Many of the latter have been pulled out of line so that several may lie in one longitudinal plane (only surface features are drawn in). Farther to the left the rows of chromomeres are

more regularly arranged and the usual fine parallel fibers predominate. It appears then that when chromomeres are pressed or pulled out of line several of their fibers come into close physical contact and produce thick compound threads not usually seen in undistorted chromosomes.

A similar condition is shown in figure 6. On the right there are two homologues, but these separate and one passes upwards (only the base of it is drawn). The other chromosome runs to the left and is greatly stretched. At the extreme left of the figure we have a heap (disarranged band) of about eight chromomeres. From these arise apparently five fibers which pass to the next disarranged band on the right. One of the fibers bifurcates just before it reaches the latter. From this second clump of chromomeres we see both fine and thicker fibers running on to the right. About the middle of the figure is a short section where the chromomeres are more regularly disposed, and at the extreme right fine lines predominate.

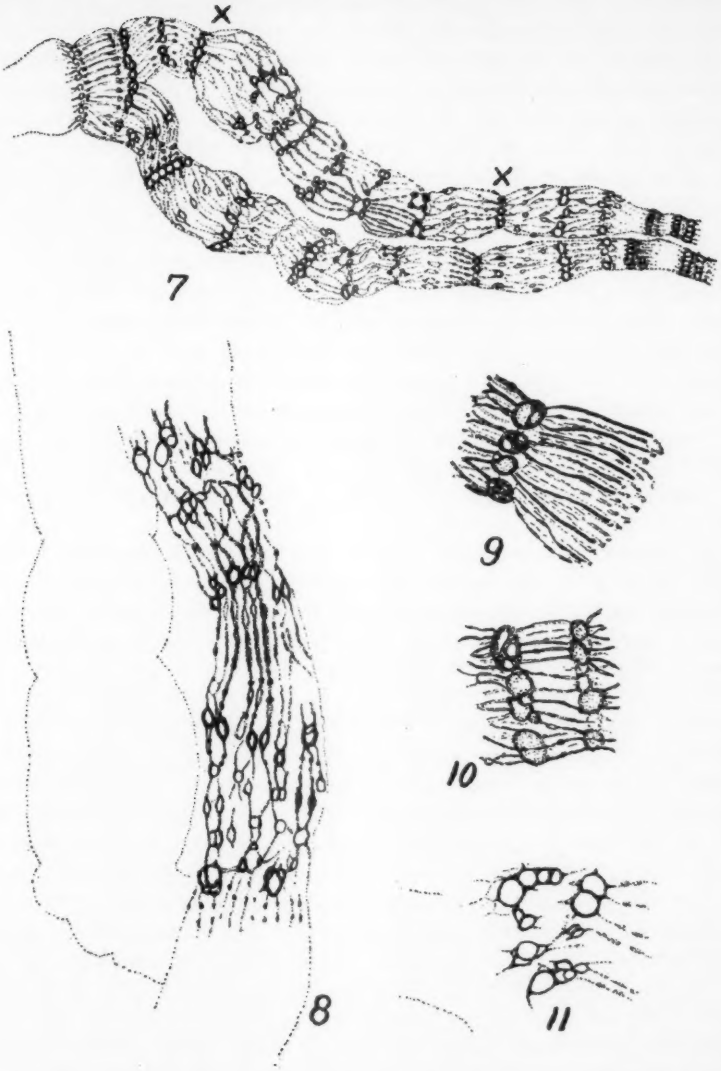
There is another cause for thick fibers, most conspicuously seen in the spread-out region, but occurring here and there in euchromatic areas. It may happen that from some cause a chromomere does not divide (or two or more fuse). From such compound elements small bundles of fibers are seen to arise.

THE STRUCTURE OF THE "SPREAD-OUT" REGION

In the spread-out region of the longest chromosome pair there appears to be little attraction between homologous chromomeres, a fact that is indicated both by a failure of the two homologues to unite in an intimate synapsis at a time when the distal euchromatic parts are closely joined, and by a lack of precision in the arrangement of the parts within a single chromatid. Here like chromomeres often form clumps instead of bands, or they may lie singly and irregularly spaced with regard to the transverse plane. This loose organization is most pronounced in a few segments; but in younger and less differentiated stages the absence of synapsis and of twisting prevents the union of the adjacent euchromatic areas so that it is only in very large elements that this region is sharply delimited.

Figure 7 is drawn at a lower magnification than most of the illustrations and represents a "half-grown" chromosome. One cannot say just where the loose organization of the spread-out area begins and ends but it is pronounced in the region lying between the X's.

Figure 8, drawn at a higher magnification than figure 7, and taken from a fully differentiated chromosome, shows surface details of one chromatid. (The other chromatid is illustrated in figure 18). At the top of figure 8 there are large, more or less oblong chromomeres with prominent vesicles and with chromatic hulls of different thicknesses. These units are inter-



FIGURES 7–11. Spread-out region. Figure 7 is drawn at a low magnification showing how the spread-out region—between the x's—is joined to the euchromatic areas. Figure 8 shows surface details of one chromatid. Figures 9 and 10 illustrate characteristic areas. Figure 11 is a drawing of part of a band which is mashed out.

connected by threads, some very fine, others quite thick and obviously compound. Some of these details can be seen in living chromosomes. About the middle of figure 8 there are thick deeply staining fibers arising from a loose band of thick-walled chromomeres above and passing downward to a more scattered transverse row of thick-walled chromomeres. These fibers seem to be made up of two or more strands, some of which bifurcate at the lower end. Below this conspicuous fibrous area we have again rounded chromomeres, many showing connections with four threads. In spite of the irregular distribution of the parts, one has no difficulty in identifying homologous elements in any general transverse plane.

The heavy fibers illustrated in figure 8 are a prominent feature of the spread-out area and, in well preserved material, at least, seem to possess considerable rigidity. The chromosome sometimes breaks just where the threads bifurcate; then the fibers stand out stiffly, like bristles in a brush. Figure 9 is a drawing illustrating such a case.

Another characteristic area of this general region is shown in figure 10. One notes that homologous chromomeres associate in clumps and vary considerably in size. Thick fibers connect these clumps in a longitudinal plane.

From the foregoing description and the figures it is evident that in the spread-out region we have the same structural elements as in euchromatic parts, namely, chromomeres and connecting fibers. But the association of homologous chromomeres in irregular clumps rather than in regular transverse rows and the apparent irregularity in the fibers, give a different and often confusing picture. Indeed, in very large chromosomes which are not stretched this region sometimes looks as if it were made up of soap bubbles or droplets of material and METZ's concept of the structure would seem to be well founded. But when the same area is elongated—the stretching of a dead and stained chromosome can do nothing more than make it easier to observe the relation of parts—the source of the confusion becomes apparent. In contrast to the euchromatic areas (where the chromomeres of a band are usually about the same size, each with its connecting thread) in the spread-out region there is a great variation in the size of homologous chromomeres. Figure 11 is part of a "band" which was mashed out by coverglass pressure. Note the differences in size of the chromomeres. The same variation can be seen at the bottom of figure 8 and in other parts, as well as in figures 9 and 10. The presence of both large and small units within one clump makes it very probable that the former are essentially compound. This might result from the fusion of two or more chromomeres, or from a failure of a chromomere to undergo fission; or possibly the union is induced by fixation. In any event, assuming that each chromomere unit lies on or is attached to a fiber, we can understand now why some fibers

are very thin while others are very thick and why a number of thin and thick fibers may appear to arise from one giant (compound) chromomere. Add to this condition an irregularity in the placement of homologous chromomeres, due to a lack of attraction perhaps, and we have all the elements needed for an understanding of the images seen under a microscope.

One more point should be stressed. When a clump of homologous chromomeres is preserved, there seems to be a tendency for the chromatin to collect at the periphery of the clump, just as it does in the compact rows in the euchromatic area. Under these conditions the contiguous walls of the vesicles are not easily made out and we observe one enormous vesicle. Sometimes tiny chromatic dots appear within these large vesicles, as BAUER (1935) has noted in the heterochromomeres of Chironomus; but if such heterochromomeres in Chironomus are essentially compound as they are in Simulium, these dots may well be either minute chromatic inclusions between the vesicular walls or portions of these walls viewed from the end.

Thus far we have made no statement about the total number of chromomeres in the bands of fully developed chromosomes, and hence the total number of chromonemata involved. Probably the type of band on which it is safest to make such counts is that containing tiny dot-like chromomeres; yet to enumerate these dots accurately is extremely difficult. From time to time we have estimated the number of chromomeres and have concluded that generally we have in the neighborhood of from 64 to 128 elements.

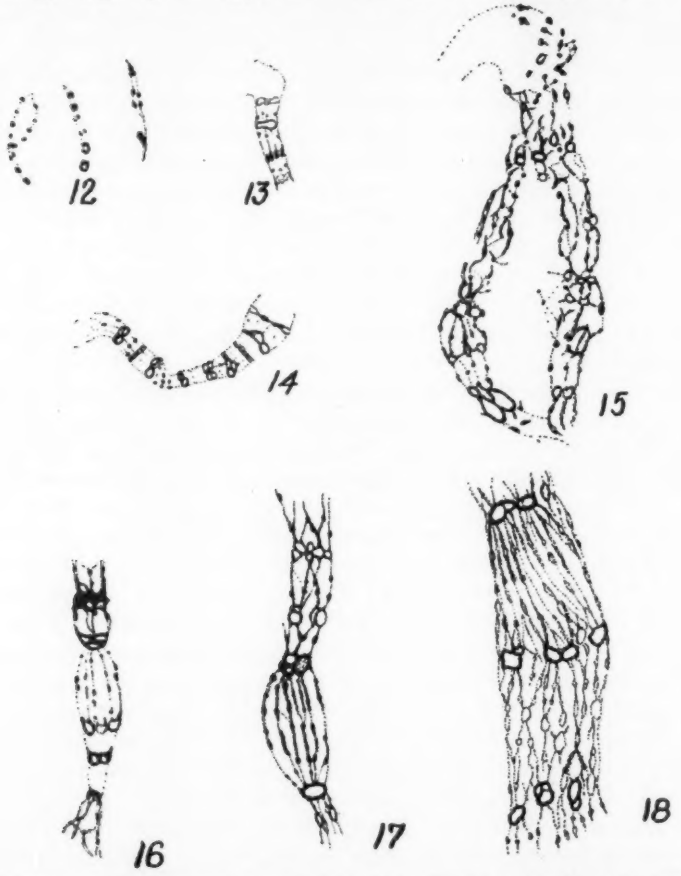
THE DEVELOPMENT OF SALIVARY CHROMOSOMES

Within a single gland there is a considerable variation in nuclear size and in chromosome differentiation, and in the glands of young larvae the differentiation can be followed from the reticular stage. For convenience we will describe the euchromatic and spread-out regions separately.

Euchromatic Area.—In the earliest stage at which we can recognize individual chromosomes, apart from the general reticulum, the nucleus is very small and usually does not rupture with coverglass pressure; hence one can follow single elements only for short distances. Figure 12 shows short sections of such young chromosomes taken from a single nucleus. We observe a series of pairs of chromomeres, part of which are connected linearly by a very fine thread. Most of the chromomeres appear as solid granules and the range in size is not very great. Since we can not recognize and follow all the individual chromosomes we do not know whether we have, at this time, single (split) elements, or two homologues which have united in synapsis. We believe the first is the case because later the twisting of the two sister chromatids is independent of the twisting of the homo-

logues (fig. 4); this is most easily understood if we assume that sister strands unite first.

As the nucleus increases in diameter the chromosomes become broader and longer (figs. 13 and 14), a condition which is obviously due, for the



FIGURES 12–18. Developmental stages. Figure 12 is the earliest stage found for incipient giant chromosomes. Figures 13 and 14 represent a 4-strand stage. Figure 15 is the spread-out region from the same nucleus as that from which figure 14 was taken. Figures 16 to 18 represent stages in the development of the same area.

most part, to an hypertrophy of most of the chromomeres and to the wider separation of the incipient bands. In figure 13 there is a maximum of four chromomeres per row and four threads; although the nucleus from which figure 14 was taken was much larger than that of figure 13, and the chromo-

somes much broader, we still find a maximum of four chromomeres per row. The hypertrophy of the chromomeres and the wider separation of the bands account for the difference in size, as the figures show. In figure 14 we note the further illuminating fact that while there are generally four chromomeres in each row there may be only three when one chromomere is much larger than the other two, or two. An example of the latter is seen in the row just below the bend in the figure. Thus it is apparent that there is some irregularity in the time of chromomeric division and certain chromomeres divide less often or less readily than others. This accounts for the condition observed in the fully grown elements, such as figure 1 or 2, where the band *d* has about half as many chromomeres as band *b* or *c*.

As we have explained, we cannot be absolutely certain that in figures 12 to 14 we are dealing with only one (split) homologue, but we think this is the case. If this is true, then between the stage of figure 12 and figure 13 or 14 there has been only one division of the chromomeres and their associated threads. If, on the other hand, somatic synapsis occurred between figures 12 and 13, no division has intervened.

In figure 6 we have a single homologue (left side of the figure) with about eight chromomeres in each row or band. Note, however, how large many of the chromomeres have become. Obviously the hypertrophy of the chromomeres has continued. Later stages show higher chromomere numbers per row and in the fully differentiated elements, such as figures 1 and 2, we estimate an average of about 64. Since we start with four chromomeric elements (the 4 chromatids) it is necessary to assume only four division cycles to account for the chromonemata observed. Most of the increase in chromosome size and nuclear volume must be due to the great hypertrophy of the chromomeres and to some extent to their greater separation.

Spread-out region.—Figure 15 is taken from the same nucleus as figure 14, and from another part of the same chromosome. Note that the two homologues are widely separated. It is difficult to make out all the fine details in the upper part of the figure, but the lower section represents all the visible elements. Here we are able to observe four chromomeres and threads, the maximum number shown in the euchromatic region (fig. 14); yet the upper part of the right hand homologue suggests that more strands may be present, a possibility which agrees with our general observation that in the spread-out region more elements appear than in euchromatic areas. Figures 16, 17 and 18 represent different stages in the development of the conspicuous fibrous area, which we can recognize very early. In the middle part of figure 16 we observe five longitudinal strands and this probably represents a transition from the four to the eight strand stage. In figure 17 we note an increase in both the length and thickness of the fibers. We can observe only six heavy fibers; but these may be compound,

for one of them bifurcates. At the top of the figure there are at least six chromomeres. Since two are larger than the others, we are safe in assuming that we have about an eight strand stage.

The end stage is represented by figure 18, which is a surface view of a single chromatid. We estimate that there must be in this one chromatid at least sixteen heavy fibers, perhaps more, which would give a total of thirty-two for a single homologue and sixty-four for the pair.

This study of development clearly shows that the great size of the fully "grown" chromosome is due to the reduplication of the original chromomeric gene string three or four times, together with a differential hypertrophy of the chromomeres and some linear extension of the threads (compare threads in figures 16 to 18).

GENERAL DISCUSSION

The bands.—From this study of developmental stages and fully differentiated chromosomes we are now in a position to understand the nature and structure of the bands seen in all salivary gland chromosomes, and to point out the bearing our findings have on cytogenetics.

During the multiplication and hypertrophy of the chromomeres within each of the chromatids, the individual sorts of chromomeres exhibit differences in behavior which account for the various types of bands observed.

1. Some types of chromomeres, usually those which show the greatest hypertrophy, do not separate completely after division, and as the number increases they become packed in transverse rows, the chromatin coming to lie mostly at the free edges where it forms a "double band" as at *b* or *d* in figure 1. There is no evidence that these chromomeres lie only on the periphery of the chromatids; on the contrary, BAUER is entirely right in his contention that the chromomeres extend through the entire diameter of the chromosome. This is plainly seen when in a mashed chromosome the "band" lies at or near a right angle to the plane of observation. The rounded surfaces of the chromomeres remind one of the surface of a honeycomb or a dish of soap bubbles.

Some workers have attempted to correlate somatic synapsis with a two by two pairing of the chromonemata, but there is no need for this assumption and no evidence for it in development. The individual homologues are split before somatic synapsis occurs and afterwards the chromomeres are held in place by the twisted condition of the separate bundles.

2. Some chromomeres divide once or twice and separate completely; then on further multiplication, the progeny of single units adhere forming a cluster of homologous chromomeres. Under these conditions the chromatin tends to lie on the periphery of the cluster, forming one "giant chromo-

mere," when we are unable to distinguish the separate vesicles (as a result of indifferent fixation or great hypertrophy). The best examples of this type of behavior are seen in the spread-out region of Simulium (figs. 8 to 10). When this occurs in the euchromatic area, there result the heavy dash-like bands which are so conspicuous in *D. melanogaster*. Needless to say, by counting these clusters we can arrive at no accurate notion of the number of chromonemata present in an element unless we know how many chromomeres are in each group.

We would interpret the "heterochromomeres" which BAUER finds in Chironomus as such homologous chromomere clusters.

3. Very small chromomeres usually separate completely giving the familiar dot-like bands which may stain deep or faintly depending on the amount of chromatin present in each unit.

Whether a given row of chromomeres will appear as a double, dash- or dot-like band will depend largely on the relative size of the units. It must be remembered that the two sister chromatids are twisted together before hypertrophy has progressed very far and there are longitudinal fibers which would tend to hold the chromomeres in place. After somatic synapsis and the twisting of homologues which accompanies it, there would be further reinforcement so that the opportunity for a movement of the units would be very limited. As growth in the size of the chromomeres and their division goes forward, the largest elements would be tightly pressed together, while the smaller ones would lie separated. If, for some reason, the usual hypertrophy did not occur in a row, the appearance of the band would be greatly altered without any further change.

Most of the bands in Simulium are compound in the sense that more than one row of chromomeres is usually present. That the same condition obtains in other Diptera is not to be doubted. PAINTER recognized the complex nature of the bands of Drosophila in his first studies and later workers have confirmed this. Thus ELLENHORN, PROKOFIEVA and MULLER (1935) were able to show, using ultra-violet light, that the second heavy band in the X chromosome of *D. melanogaster* is composed of at least four rows (discs) of chromomeres. This complex nature of the bands has, of course, a very direct bearing upon a number of the current problems in cytogenetics.

Every one who has worked on *D. melanogaster* salivary chromosomes has doubtless encountered difficulty, at times, in identifying in a given region of a chromosome the bands shown on the chromosome maps of PAINTER or of BRIDGES. Aside from variations due to fixing and staining and incomplete or poor illustrations, different degrees of stretching often entirely alter the appearance of a familiar and a generally conspicuous band. Prolonged study of any restricted area, with many preparations, invariably

shows more bands than we first thought present and, for this reason, the exact localization of gene loci is not as easy as it first appeared. Earlier estimates about the number of genes carried by the species, on the assumption that each band represents a gene, have required revision. Thus BRIDGES' first estimate of some twenty-six hundred has given way to five or six thousand (cited from BAUER 1937) and MULLER and PROKOFIEVA (1936) reckon the number now around ten thousand. Unless we know how many genes are represented, it is hazardous to calculate the size of the individual gene from measurements on salivary chromosomes. In studies dealing with the patterns of related species it is worth while to remember that should a break come between the elements of a compound hand, a familiar landmark would disappear. Also, the failure of similar chromomeres to undergo the same degree of hypertrophy, in two species might be very misleading.

Our observations indicate that one cannot judge the composition of a band unless the individual chromomeres can be seen. A double band may represent one row as at *b* or *d* in figure 1, or three rows as at *c*, *f* and elsewhere in this figure. In a recent paper, METZ (1937) has pointed out that in a stock of Sciara which has a deficiency in one homologue, only one half of a "double band" is missing; he uses this as an argument against the chromonema theory on the implied assumption that this double band is made up of one row of chromomeres. Needless to say his evidence can not be considered critical until he shows that only one row of chromomeres is involved.

The Chromomeres.—In the youngest stage, the vast majority of the chromomeres appear as solid chromatic granules, not very different in size. But as the chromomeres begin their differential growth or hypertrophy the range of sizes may become very great especially in chromosomes of the large vesicle type. Figure 1A shows, in a semi-diagrammatic way, the apparent structure of twenty-eight different chromomeres. Here we note that all but the smallest are vesiculate, and there is no reason to suppose that they are different in structure. The chromatic hull may be thick or thin, evenly or asymmetrically distributed about the vesicle. The non-staining vesicles, in turn, may be relatively large or small, and when the chromatic hull is very thin, as often obtains in parts of the spread-out region, it may be difficult to distinguish between vesicular and interchromomeric spaces. Since a given chromomere, under the same conditions, generally gives the same form expression, the variations observed must be due to intrinsic and not external causes. The relative amount of chromatin in the vesicular wall, the way it is distributed (aside from external pressure), whether the hypertrophy is limited or marked, all are properties of the particular chromomere concerned. We now turn to discuss some questions which are raised by our observations.

Foremost stands the question, how are the chromomeres observed at very early stages (for example, in figure 12) related to those seen in fully differentiated chromosomes. To answer this question with finality, it would be necessary to make detailed comparisons between whole chromosomes, at the initial and the final stages. This has not proved possible so far. We regard it as very probable, however, that the chromomeres seen at first (fig. 12) are compound, in the sense in which BELLING (1928) used this term, that is, are made up of several different kinds of chromomeres. The simplest way would be to regard the early chromomeres as corresponding to all of the elements of several closely associated bands. Thus in figure 1, there are seven rows of chromomeres closely associated in the region of *b*, and no doubt these would all appear as one in the early stage. Likewise all the chromomeres clustered about rows *e* and *f* would probably look like one large chromomere. As the individual chromomeres of these aggregates begin to grow, they cause the chromosome to increase in length as well as in diameter and the component parts become visible. At this point it is well to remember that in figure 1, the fixed chromosome is stretched and in the living state the bands would be much closer together.

If we have correctly interpreted the facts, the chromomeres of single rows would correspond to the "ultimate" chromomeres which BELLING saw in the liliaceous plants and it is interesting to note that there are several points of similarity in their fundamental structure. The "gene chromatin" is the chromatic hull, and while BELLING does not mention it he shows a nonchromatic space, corresponding to our vesicles, between the rind and the submicroscopic dot which he thought might be a "naked gene." BAUER (1936) has described and figured a number of tiny chromatic dots in the heterochromomeres of Chironomus and we have seen them in large chromomeres (figs. 9 and 10) of Simulium; but these large chromomeres are almost certainly homologous chromomere aggregates and the dots may be simply explained as points where the abutting walls of vesicles enclose bits of chromatin, most of which is pressed to the periphery of the cluster. We may well ask whether the dots seen by BELLING may not be explained in the same way. Not all cytologists will agree that the leptotene threads are single; indeed, there is much cogent evidence to the contrary (NEBEL and RUTTLE 1937).

Another interesting feature of this study is the demonstration that the simple longitudinal division of the original (4) chromatids four times does not account for the enormous increase in chromosome size and nuclear volume. Most of this increase is the result of the hypertrophy of the individual chromomeres. That this hypertrophy is associated with the great secretory activity of these gland cells seems probable but how is the increase in the chromatin and vesicular material brought about? Several possibilities suggest themselves. One might suppose that the large size of

many of the chromomeres was due to the accumulation of some sort of secretory material elaborated by the genes. Or, as seems more probable at present we may say that the hypertrophy is due to a growth or reduplication of the chromomeric substance without a visible separation into smaller units. Several lines of evidence indicate that this may be the explanation. (1) In spite of the great size of Simulium chromosomes, there is evidence for only about 64 chromonemata. In contrast to this, in Chironomus BAUER finds evidence for 350–400 strands. And in *D. melanogaster*, where the number of chromonemata is variously estimated as 16, 32 or even 64, HERTWIG (1935) has calculated that on the basis of nuclear volume, there should be at least 512 strands. These discrepancies can be readily explained if we assume that the chromomeres seen in many forms are really homologous chromomere aggregates and have often high genic valences. In Simulium, in young stages there may be four, three or two chromomeres in adjacent rows of the same chromosome. If there are three, one is characteristically larger than the other two, indicating a growth of the chromomeric substance without a division. And elsewhere we find abundant evidence that many of the chromomeres have higher valences than one. Furthermore, the longitudinal threads seen connecting the chromomeres in fully developed chromosomes generally appear tubular, a fact which we can well understand if they really represent bundles of thinner strands.

We do not propose to discuss, in detail, METZ's ideas of chromosome organization and the objections he has raised to the chromonema concept. Many points have been covered in the body of this paper, and the evidence from the ontogenetic stages leaves little room for doubting the correctness of the original suggestion of BRIDGES and of KOLTZOFF. We follow the current practice of calling the separate longitudinal elements, chromonemata, not in the first sense of the word "chromonema," implying as it does a more or less uniformly thick thread, but rather as the separate units (daughter gene strings) which are found within one apparently single chromosome. In reality, these gene strings have a chromomeric structure and we believe this must be considered the fundamental structural organization of all chromosomes, other conditions being derived from this.

Morphologically a chromosome consists of chromomeres which are held together by a longitudinal filamentous strand of protoplasm; the chromomeres consist of an outer covering of chromatin and an inner space filled with achromatic material. This concept is not new; the thread, the chromatic rind and the vesicle have all been described and figured repeatedly. Neither the thread nor the vesicular material stains appreciably with aceto-carmine, and whether the thread is really separate from, or a prolongation of, the vesicle must remain an open question.

New light has recently been thrown on the physical and chemical nature

of chromomeric chromosomes by CASPERSSON (1936), who has approached the subject from a different angle. CASPERSSON developed and applied to the salivary chromosomes of Chironomus a technique for the study of the chromatin, involving the use of ultra-violet absorption curves and a special enzyme which digests away the proteins, leaving the chromatin as an insoluble lanthanum salt. He finds that the chromatin is in the form of nucleic acid. When the proteolytic enzyme is used, the first part of the salivary chromosome to be affected is the thread region which quickly dissolves, indicating that these strands are protein in nature. Initially the vesicular material of the chromomeres is unaffected, but on prolonged digestion this material also dissolves indicating that it too is protein. On the basis of CASPERSSON's work it appears that we have three types of substances in the salivary gland chromosomes, a conclusion which is in complete agreement with the cytological picture in Drosophila (BRIDGES and others), Chironomus (BAUER) and Simulium. Of course it is possible that the thread and vesicular material may be one and the same substance, for naturally an extremely thin thread of protein would be more quickly affected than the relatively massive vesicles.

We must now consider the question "Where is the locus of the gene?"—a problem necessarily bound up with the ultimate structure of the chromosome. Some progress may be claimed for the present study, not in that the individual facts are new, but rather because Simulium chromosomes are so favorable for study that the chromomeric nature of chromosomes rests on a broader and firmer foundation, the essential morphological parts or components standing out, perhaps, in sharper focus. With one notable exception (METZ) most cytogeneticists who have recently considered the gene's locus have assumed a chromomeric organization, and we find expression of the opinion that (a) the genes are represented by the chromomeres in some way; (b) that the interchromomeric threads contain the genes; and (c) that the whole chromosome is made up of genes end to end, there being no intergenic material; in short, that both the thread and the chromomere may represent a gene complex. A brief review of these ideas will serve to give the status of the question at present.

While many cytologists have doubtless thought of the chromomeres as representing the genes, BELLING brought this view into the foreground, from his analysis of liliaceous plants and his concept of an "ultimate chromomere." Of course, BELLING had no real evidence that the chromomeres contained the genes except that the genetic linear order of the genes had a striking counterpart in the linear differentiations of the chromosome. When later it was found that definite chromomeres along the salivary gland chromosomes of *D. melanogaster* could be associated with definite genes, PAINTER regarded the chromomeres as the probable locus of the

genes, a view subsequently shared by many Drosophila workers. But it was, and is, realized that the evidence is not unequivocal because the absence of a band, and with it a gene, does not exclude the possibility that the gene lies outside of the chromomere and on the thread, part of which is removed with the band. In view of our experience with Simulium we may well question whether any of the deletions studied by Drosophila workers consist of a single row of chromomeres. Rather it is the fact that the chromomeres are arranged in a linear order and that they alone show great qualitative differences, such as one might expect from the varied nature of the genes, which has caused the Drosophila workers to look to the chromomere as the gene's home. It is interesting to note that CASPERSSON has been led to the same general point of view, for while he thinks that the protein of the thread might represent enough varieties of molecules to account for the genes, he points out that the compounds which could be formed within the chromomeres, between the protein and the nucleic acid, would give a much wider range and variety of chemical combinations; for this reason the chromomere might be regarded as the more probable locus of the gene.

The view that it may be the achromatic portion of the chromosome which carries the gene has been advocated by KOLTZOFF (1934) and seems to be favored by METZ. Here let it be noted that KOLTZOFF referred to the interchromomeric threads, while METZ, who envisions another type of chromosome structure than that accepted here, would include both the thread and the achromatic portion of the vesicles, in terms of the present paper. We think that cytogeneticists generally recognize the possibility that the genes might lie between the chromomeres, but at present this seems less probable.

From a theoretical genetic standpoint MULLER has pointed out that a chromosome might be made up of a chain of genes linked end to end without any intergenic substance or thread. The same sort of structure is implied by WRINCH'S (1935) theoretical concept of salivary gland chromosomes. The latter writer approaches the problem from the standpoint of molecular structure and pictures the chromosome as made up of amino acid units linked in linear order. Some of these units are basic; and to these the nucleic acid molecules become attached, forming the chromatic deposits or bands. When the amino acid unit is not basic, no nucleic acid will be deposited. The genes, being specified as amino acid units, may lie either in the banded or in the achromatic (thread) region.

From this brief review, it is evident that while there is as yet no unanimity of opinion about the gene's locus, nevertheless we are passing from the theoretical speculative stage to a vigorous practical attack upon

the problem from a number of different angles, an attack made possible by a better understanding of chromosome structure.

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A TANDEM INVERSION IN DROSOPHILA MELANOGASTER

MARGARET E. HOOVER

Carnegie Institution of Washington, Cold Spring Harbor, New York.

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INTRODUCTION

BY MEANS of X-rays it is possible to procure gene deficiencies and chromosomal aberrations in Drosophila which can be analyzed genetically and cytologically. The increasing refinement of technique in these fields is facilitating such detailed parallel studies. As a consequence there arises an ever-growing mass of problems concerning such questions as the origin of the changes and the relationships involved. In order to have a better understanding of these relationships we need to determine the types of changes which are occurring, and an analysis of different kinds of alterations may aid in a more complete understanding of the underlying mechanism involved. The present analysis was undertaken to obtain information about the relationship between chromosomal aberrations and deficiencies.

MATERIAL

With the intention of picking up lethal changes connected with known loci along the X chromosome of *Drosophila melanogaster*, *y Hw* males were treated with X-rays using a dosage of approximately 2500 r units. Following the treatment, these males were mated to females of the constitution *ct v dy g f*/In dl–49, *y Hw* m^2 g^4. The F_1 flies of this cross were examined and all females were saved which besides being heterozygous for *Hw* exhibited one of the mutants carried by the female parent. In the particular instance described in this paper an F_1 female was found with cut wings. The male off-spring of this female were only half as numerous as expected, and hence she was assumed to carry the cut mutant in the maternal chromosome, and either a lethal mutant for cut or a cut mutant plus a lethal in the paternal chromosome. Crossover tests were made by mating *y Hw* ct^1/*sc ec cv* ct^6 *v* s^2 *f car* bb^1 females with *ct v dy g f* males. No crossovers were observed among 141 males and 1007 females. This suppression of crossing over thus indicated that in addition to the change in the cut locus and a lethal which might or might not have been connected with cut, the treated X chromosome was involved in some chromosomal rearrangement. Therefore, the location of the lethal could not be determined by genetic means. Since it is known that lethals may be deficiencies (DEMEREC and HOOVER 1936; ALIKHANIAN 1937), salivary gland chromosomes of female larvae were studied cytologically in an attempt to determine the position of the lethal. The study revealed two inversions in the X chromosome—a

small one involving approximately 4.5 sections of the cytological map prepared by C. B. BRIDGES (BRIDGES 1935), and another larger inversion involving 11.5 sections. There is no uninverted portion of the chromosome between the two, so that we have here a case of what may be called inversions in tandem. This terminology may be conveniently used to designate two inversions, one following immediately upon the other in the same chromosome.

METHODS

In order to study this material, slides were prepared of the salivary gland chromosomes of female larvae according to the aceto-carmine method and were made permanent by the alcohol-euparol technique described by BAUER (1936). In analyzing the slides the optical equipment consisted of a 90X, 1.4N.A. apochromatic objective, an oil-immersed achromatic 1.4N.A. condenser and compensating oculars (12.5 and 15X). The source of illumination was a Bausch and Lomb research lamp (ANTHES 1936, BRIDGES 1936) equipped with a Wratton filter number 58A.

To guarantee the greatest accuracy in determining the breakage points of the inversions, observations were made on two types of figures, synapsed chromosomes and single haploid strands. The synapsed diploid chromosomes were studied to determine the homology of the bands present. However, in the complexity of even a very simple aberration, it is not easy to identify accurately the bands immediately bordering a break, so that confirmatory information must be obtained from stretched haploid strands. In these unsynapsed strands the band can be identified only by its relative position and intensity. Those working with similar problems are well aware of the risk involved here, for the amount of stretching of the chromosome and the particular quality of the illumination used may alter the general appearance considerably. Consequently in such an analysis as is attempted here, it is fair only to make suggestions as to what the conditions may be, suggestions based on as accurate statistical observations as were feasible.

RESULTS

The two inversions in heterozygous condition appear in the salivary gland chromosomes as two loops when both strands are completely synapsed. Complete synapsis occurs in 77 percent of the cells and complete asynapsis in 3 percent. By complete synapsis is meant the pairing of the two strands throughout their entire lengths with the possible exception of a very small region at the junction of the two loops, a region for which complete pairing would be quite difficult to determine. By complete asynapsis is meant the two strands separate throughout their entire lengths. This 77 percent may be compared with 90 percent complete synapsis for controls (X chromosomes not containing inversions), and the 3 per-

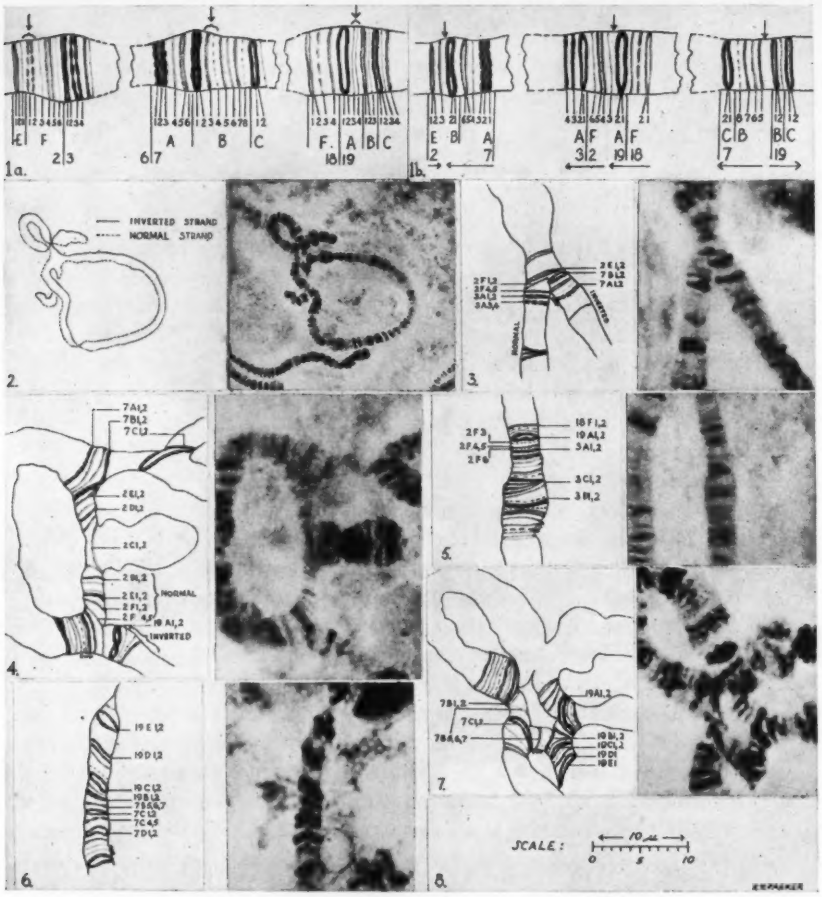

FIGURE 1a. Map of the salivary X chromosome showing normal banding in regions discussed in this paper. (Prepared by C. B. Bridges.) Arrows indicate breakage points and brackets, deficiencies.

FIGURE 1b. Same regions showing sequence of banding in tandem inversion.

FIGURE 2. General figure of the tandem inversion in the X chromosome showing the loops produced by synapsis of the homologous loci of the normal and inverted strands. The accompanying sketch shows the direction taken by each strand.

FIGURE 3. The first breakage region. The first bands of the first inversion are 7B1, 2. The asynapsis permits comparison of the order of banding in the normal and inverted strands.

FIGURE 4. The first breakage region showing 2F3 followed by 7B1, 2 and the second breakage region showing 2F3 followed by 19A1, 2, thus indicating a deficiency for 2F1, 2.

FIGURE 5. Haploid inverted strand showing the second breakage region with 2F3, the last band of the first inversion, followed by 19A1, 2, the first bands of the second inversion.

FIGURE 6. Haploid inverted strand showing the third breakage region with 7B5, 6, 7, the last

cent of complete asynapsis may be compared with 1 percent for controls. Similar observations have been made on six other inversions of differing lengths in the X chromosome (HOOVER 1937). Although the longest inversions (ClB and the tandem inversion) show the lowest percentages for complete synapsis, yet there is indication of very little, if any, correlation between inversion length and synaptic attraction.

When complete synapsis does take place, the inversions appear as indicated in figure 2. In each inversion one strand is inverted in relation to the other, so that, as the diagram shows, when pairing occurs, loops necessarily result. The two inversions use in their combined configurations almost the entire X chromosome leaving only small sections at each end in which both homologous strands are in their normal positions. There are actually two separate and distinct inversions, and, as the order of banding clearly reveals, not one inversion imposed upon or overlapping the other.

Three breaks in the X chromosome may account for the origin of these two inversions since one breakage point is common to both of them. In figure 1a are given diagrams of the three regions as they normally appear under optimal conditions of stretching. These normal maps have been furnished by Dr. BRIDGES as revisions of his 1935 maps (BRIDGES 1935). The first break, which is the left break of the first inversion, precedes 2F3; it follows 2E3. The second break in the chromosome which is common to both inversions follows 7B1, 2 and precedes 7B5. The third break follows 19A1, 2 and precedes 19B1, 2. Therefore, the first inversion extends from 2F3 to and including 7B1, 2 and the second inversion extends from 7B5 to and including 19A1, 2. The normal portions extend from the tip to and including 2E3 and from 19B1, 2 to the spindle fiber locus.

In figure 1b are represented these regions as they are recombined by the inversions. The 2E3 section thus comes in contact with the 7B2, 1 region, 2F3 comes in contact with 19A2, 1, and 7B5 with 19B1, 2. Since the critical lines at these breakage points are very fine, it has not been possible to obtain photographs of the figures which show them. Photographs and camera lucida drawings are given here only of figures which were reasonably good for reproduction. Figure 3 represents the first breakage region. The last synapsed band visible in that figure is 2E1, 2. Other figures have clearly shown that a fine line 2E3 completes this first normal section and that, as shown here, 7B1, 2 begins the first inversion. Comparing the two

bands of the second inversion followed by 19B1, 2, the first bands in the normal portion at the end of the chromosome. The absence of other bands between 7B5, 6, 7 and 19B1, 2 makes it probable that there are deficiencies for 7B3 and 4 and for 19A3, 4.

FIGURE 7. Third breakage region with 7B5, 6, 7 followed by 19B1, 2.

FIGURE 8. Scale referring to figs. 3 to 7.

Photographs and drawings by Rachel W. Parker.

strands, one normal and one inverted, the first inversion would seem to follow upon 2E3. Figure 4, however, shows that 2F1, 2 is not present at the other end of the first inversion where it would be expected to be if the breakage occurs following 2E3. Figure 4 again shows not only that 2E3 is followed by 7B1, 2 but moreover that 2F3 is followed by 19A1, 2 and, accordingly, there is a deficiency at this breakage for 2F1, 2. These two figures also show that 7B3 and 4 and 19A3, 4 are not present at these breakage points, making it necessary that all four of these bands be detected at the third breakage region if they are present at all. The second breakage region is illustrated in figure 5 showing an inverted haploid strand where it can be seen that 2F3 is immediately followed by 19A1, 2. It is clear from that figure that there is no band between 2F3 and 19A1, 2, as has already been indicated in figure 4. Finally, in figures 6 and 7 is illustrated the third breakage point. In figure 6, which again shows a haploid inverted strand, bands 7B5, 6, 7 are followed by 19B1, 2. Close examination of this and many other figures has failed to reveal any suggestion of a band or bands between. Similarly, in figure 7, 7B5, 6, 7 appear as the final bands of the second inversion and the normal stretch at the end of the chromosome begins with 19B1, 2. These observations therefore suggest deficiencies for 7B3 and 4 and for 19A3, 4. Negative evidence of this type is not entirely satisfactory. In the present instance, however, numerous well-stretched figures were examined without any sign of the bands in question, so that it seems probable that small deficiencies have occurred at each of the breaks. The following appears then to be the condition at hand:—*normal:* 1A1–2E3; *deficient:* 2F1, 2; *first inversion:* 2F3–7B1, 2; *deficient:* 7B3 and 4; *second inversion:* 7B5–19A1, 2; *deficient:* 19A3, 4; *normal:* 19B1, 2–20D.

DISCUSSION

One of the interesting problems connected with the study of chromosomal aberrations is to determine whether all chromosomal aberrations have small deficiencies connected with them. It is known that all deficiencies are not connected with other chromosomal alterations; it is also known that all chromosomal aberrations are not connected with lethals; but since it is being found that some deficiences are not lethals (DEMEREC and HOOVER 1936), it remains to be determined how many translocations and inversions are free from accompanying deficiencies.

The available data for the relationship of gene changes and chromosome rearrangements are recent. Although observations have been made on numerous deficiencies and numerous aberrations, statistical data on the interdependence of the two are just being accumulated. The existence of such an interrelationship is indicated from results obtained by DEMEREC

(1937) who found that of 61 lethals induced in known X chromosome loci by X-ray treatment, 26 carried a chromosomal aberration in the same chromosome and that in all but one case one breakage point of that aberration coincided with the region where the lethal change occurred. Of 30 visible changes induced by a similar treatment in the same set of loci only one chromosomal aberration was observed and in that case neither of the breakage points coincided with the region of the visible change. Since it is probable that lethal changes may be deficiencies, this suggests a close relationship between deficiencies and chromosome breaks. The present material gives additional evidence on this same problem. The two distinct inversions have apparently one breakage point in common at the cut locus which was simultaneously changed, and there are indications of a deficiency at each of the three breakage points.

Genetic evidence shows that the tandem inversion is lethal when homozygous. It has not been possible to localize the position of that lethal in relation to cut. There is every indication of a cytological deficiency in the cut region as well as deficiencies at two other places in the chromosome. It is interesting to note that tests applied to the hypodermal cells of females indicate that the cumulative effect of all the deficiencies is non-cell-lethal, an effect which has been limited to lethals of a very few of the known loci tested (DEMEREC 1934). In this respect the lethal here resembles other lethals at the cut locus.

The question naturally follows as to how these inversions and deficiencies have originated. Two alternative hypotheses are possible. One is that the X-ray treatment produced three breakages along the chromosome and that the broken pieces so rearranged themselves as to become fused together in a new arrangement. On the other hand, it is possible to visualize the chromosome as looped upon itself so that fusion of overlying strands occurred at the time of treatment, and following this fusion, breakages occurred resulting in a rearrangement of the gene order. The deficiencies which result would be conceived of as coincident with the breakages so that, as has already been suggested, the mechanism responsible for these abnormalities is also frequently responsible for lethal deficiencies. This material does not offer critical evidence for choosing between these two alternatives.

SUMMARY

A tandem inversion involving practically the whole X chromosome was obtained following X-ray treatment. Genetically this material involves a change in the cut locus, and a lethal, which while lethal in the homozygous condition to the organism as a whole, is non-cell-lethal to hypodermal cells of females. Cytologically three points of breakage have been determined

in the salivary gland chromosomes, and the heterozygous chromosomes have been observed to form two characteristically looped inversions. The two inversions have a common breakage point following 7B1, 2 and preceding 7B5. The first of the two other breaks occurred following 2E3 and preceding 2F3, and the second follows 19A1, 2 and precedes 19B1, 2. Cytological examination indicates that small deficiencies have occurred at each of the three breakage points.

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MOTTLED WHITE 258–18 OF DROSOPHILA MELANOGASTER

M. DEMEREC AND HELEN SLIZYNSKA[1]
Carnegie Institution of Washington, Cold Spring Harbor, New York

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IN THE early experiments with X-rays it was noticed that mottled types frequently appear among the offspring of treated parents. MULLER (1930), in describing several of these types, ascertained that mottling is connected with chromosomal aberrations such as translocations and inversions, and SCHULTZ (1936) found that the locus involved in mottling is brought into proximity of the inert region through chromosomal rearrangements. This observation has been confirmed by DUBININ (1936) and SACHAROV (1936).

Several hypotheses were offered to explain the mechanism of mottling. PATTERSON (1932) made an attempt to account for the appearance of two kinds of tissues by assuming somatic loss of a piece of chromosome through unstable translocation; STERN (1935) assumed a rather special type of duplication undergoing frequent somatic crossing over; and SCHULTZ (1936) suggested that mosaicism could be explained through the formation of a chromosome ring and subsequent dropping out of small pieces from such a ring during somatic division.

Stocks which are available in this laboratory for showing induced changes include six white mottled types. Out of these we selected for our study type number 258–18 which is viable in males and shows what seems to be a simple variegation pattern. We chose to begin our work with the simplest type available and thus obtain evidence which would be helpful in the analysis of the more complicated forms.

The senior author was in charge of the breeding work and the junior was in charge of the cytological phase of the problem.

Origin. The 258–18 mottled was found in November 1935 as a single female which came from a mating between a white-miniature-forked female and an X-rayed yellow male. Therefore, the chromosome in which mottled appeared also carried yellow. Mottled males and homozygous mottled females are viable and such flies also show the eye character roughest, an allele of roughest[2].

EXPERIMENTAL DATA

Cytology. The study of salivary gland chromosomes shows that 258-18 is a reciprocal translocation between the X and the fourth chromosome.

[1] Guest investigator; Research assistant at Animal Breeding Institute, University of Cracow, Cracow, Poland.

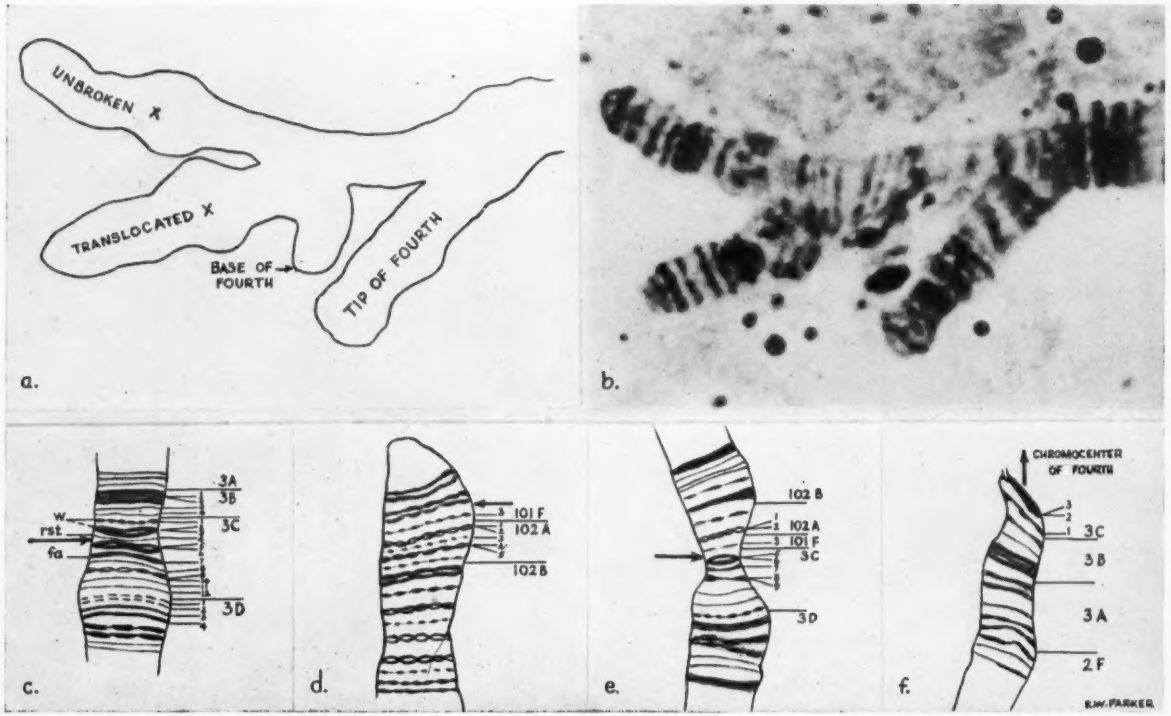


FIGURE 1. a and b. Outline drawing and photograph of a salivary chromosome configuration common in females heterozygous for 258–18. The tip of the fourth chromosome is attached to the X at $_3C_5$ and the tip of the X is attached to the chromocenter of the fourth.

c and d. Diagramatic drawings of sections of the X and the fourth in which breaks have occurred, arrows indicating breakage points (after C. B. BRIDGES).

e and f. Camera lucida drawings showing regions of fusion between the X and the fourth.

The break in the X is between bands 3C3 and 3C5 and in the fourth preceding band 102A1. Figures 1a and 1b show an outline drawing and a photograph of one of the salivary chromosome configurations common in females heterozygous for 258–18. The tip of the fourth is shown to be attached to the X, and also the tip of the X is attached to the chromocenter of the fourth. The non-translocated chromosomes shown in this figure carry the Notch–8 deficiency of MOHR. Figures 1c and 1d are diagrammatic drawings of sections of the X and the fourth in which breaks have occurred, arrows indicating breakage points. Figures 1e and 1f are camera lucida drawings of salivary chromosome figures showing regions of fusion between the X and the fourth.

Band 3C4 (fig. 1c) is a very fine band which can be seen only under the most favorable conditions. During an intensive study of this region of the chromosome made by SLIZYNSKA, the 3C4 band was seen in only one figure. It was not seen in the 258–18 material. Indirect evidence, however, indicates that this band probably represents the roughest locus. GRÜNEBERG (1937) has shown that roughest is located 0.2 units to the right of white and therefore in the region between white and facet. The work of SLIZYNSKA (in press) indicates that the white locus is probably represented by 3C1 and the facet locus by 3C7. This work shows also that the deficiency for 3C2, 3 does not involve roughest. This indicates that roughest is between 3C3 and 3C5, namely, that it is represented by 3C4 band. Since the break in 258–18 occurred after roughest, it seems probable that it occurred between bands 3C4 and 3C5.

Description. On the eyes of homozygous 258–18 flies, cream, cherry and red colors, with both roughest and smooth facets, appear in definite patterns. In the line with light-colored eyes the background color of the eyes is cream. On this background cherry and red spots ranging in size from one facet to many facets may be present. If a cherry spot covers a large sector, a smaller red spot may be found on the cherry background. Cream and cherry colored regions are always roughest. On red-colored sectors, smooth spots may be present on the roughest background. In figure 2a, spots appearing on light colored eyes are shown diagrammatically. Color mottling is detectable also in integuments of the testis and in Malpighian tubules.

By selection, a line has been isolated in which all flies have dark-colored eyes. Such eyes have a red and smooth background on which roughest spots may appear. Cherry and cream spots appear only on roughest sectors. Figure 2b shows diagrammatically the type of spotting which appears on dark-colored eyes.

Breeding results. The type of spotting in 258–18 translocation may be readily influenced by various genetic and environmental factors. By

continuous selection, light lines and dark lines can easily be isolated presumably through sorting out of different types of modifiers. The influence of genetic environment is best expressed in the behavior of 258–18 in combination with the white allele. Red spots on $w\ w^{cr}$ females are much larger than on either $w^{cr}\ w^{cr}$ females or w^{cr} males. Eyes of females of $w\ w\ w^{cr}$ constitution are almost red. The 258–18 is affected by the Y chromosome as it has been already shown for some other mottled characters (GOWEN and GAY 1934; SCHULTZ 1936). The addition of an extra Y chromosome produces a near wild type phenotype both as far as color and roughest are concerned.

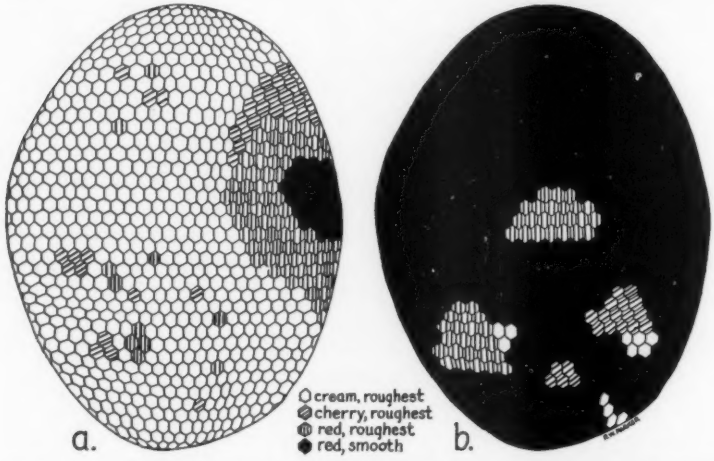


FIGURE 2. a. Diagram showing spots appearing on light-colored eyes where background is cream and roughest.

b. Diagram showing spots on dark-colored eyes where background is red and smooth.

Temperature also influences 258–18 mottling. Flies raised at 29°C have more wild type tissue on the eyes than their sibs raised at 25°C.

As has been mentioned previously, three colors are evident on the eyes of the 258–18 flies, namely, cream, cherry and red (wild type). On homozygous flies the demarkation line between different colored sectors is sharp. There is no evidence of intermediate colors. In the course of experiments with the 258–18 material, a large number of homozygous flies was examined and among them one male was found which had white mottled instead of cream mottled eyes. It was yellow like its parents, which eliminated the possibility of contamination from another white-mottled stock available in the laboratory at that time. The male offspring of that white-mottled male by attached-X female were white-mottled and thus a line

has been established which has the background color of the eyes white instead of cream. In this line the relationship between white, cherry, red, roughest and smooth spots is similar to the relationship between cream, cherry, red, roughest and smooth spots in the cream line.

DISCUSSION

In order to explain the mechanism responsible for mottling it is essential to have a clear picture of the sequence of changes producing various patterns. It is fortunate that in the homozygous 258–18 flies the outline of the spots is sharp, thus greatly facilitating the analysis of this case.

Flies of the light strain have cream-colored eyes with a small number of dark spots, which may be either cherry or red. These spots are usually small, extending over one to ten facets, and are distributed at random over the eye. Eyes with as few as one to five such small spots are easily found. When spots are more numerous, some of them cover larger sectors, but their distribution is again at random. Eyes with as many as 30 to 40 spots are common. The distribution of these spots indicates that eyes of the light line began their development as cream and that the dark colored spots originated late in ontogeny through changes from cream to dark color. The other possibility, that eyes start their development as red and through early changes to cream develop large sectors of cream tissue, is made improbable by the distribution of spots. In order to explain on this basis the observed distribution of spots, it would be necessary to assume that eyes do not develop through a regular cell lineage. That this is not the case is shown by the appearance of medium-sized spots which cover definite sectors indicating a high degree of regularity in cell lineage. The pattern of the spotting on light-colored eyes, therefore, indicates that cream may change into darker colors.

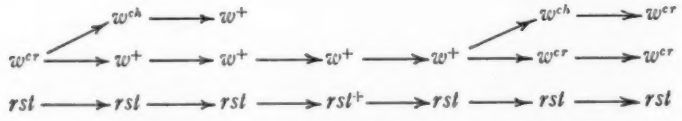
Since two dark colors, cherry and red, are distinguishable, the question arises as to the relationship between the changes producing them. Is cherry caused by a change affecting only a few cells of one facet or, if that is not the case, does a change to red regularly go through cherry? Since a cherry spot may cover a sector involving a large number of facets, it is evident that cherry can not be red diluted through the development of color in only a few cells of a facet. Also, red spots frequently appear as single facets, which indicates that cream can change directly into red. That cherry can change into red is indicated by red spots appearing on cherry sectors. Cream, therefore, can change directly into red or it can change first into cherry and cherry into red.

In addition to colored spots, mottling affecting texture of the eye (roughest and smooth) is evident on the eyes of the 258–18 flies. This mottling is due to changes affecting the roughest locus which is situated next

to the breakage point in the X chromosome. Smooth spots never appear either on the cream or on the cherry background. On light colored eyes smooth (rst^+) spots are found only on red sectors. In this case, therefore, the direction of change is from roughest to smooth, but in order that this change may occur it is essential that cream should first change into red.

In dark lines, which can be obtained either by selection or by raising flies at high temperature, the eyes may be entirely red and smooth; may have roughest spots; or may show roughest, cherry and cream spots. Cherry and cream spots appear only on roughest sectors. The type of spots indicates that, in this case, the direction of change is from smooth to roughest, and from red to cherry or cream, the latter appearing only on roughest sectors.

The study of spotting suggests that changes in the case of 258–18 occur in both directions, from mutant into wild type and also from wild type into mutant and that changes in the white and the roughest loci are interdependent. The sequence of these changes is shown in the following outline:



These changes in white and roughest are frequent. They can always be observed on individuals capable of showing them. Only once, so far, was a male found which had white instead of cream colored eyes with cherry and red spots. This male is the progenitor of a line which shows the same type of spotting as the line described above except that it has a white background instead of cream. In this case, apparently, a change to white has occurred at the white locus which did not affect other changes occurring at the same locus. That this white color is not the result of the interaction of cream and another color such as garnet or carnation is shown by the fact that dark spots are red, similar to these found in the cream line, and not garnet, carnation or some other mutant type.

After this consideration of the changes occurring in 258–18 mottled, the mechanism responsible for these changes may be considered. All explanations offered for similar mottling assume that the loci involved became homozygous in part of the somatic cells. According to PATTERSON (1932) this is accomplished through a loss of the translocated piece; according to SCHULTZ (1936) through a loss of a small section of the chromosome, and according to STERN (1935) through somatic crossing over. All these hypotheses require as a starting point either a heterozygous or a dominant condition and can explain only changes occurring from the dominant into

recessive phenotypic expressions. Since, in our case, changes not only occur in both directions but also three alleles of the white locus (cream, cherry and red) are involved, none of the hypotheses offered can account for the behavior of 258–18. In addition, the crossing over hypothesis can not account for the spotting in males which are haploid for the X chromosome, and according to both of the "loss" hypotheses, white spots would be expected and not cream ones, as observed here.

However, a large group of genes is known which in many respects parallel the behavior of 258–18. These are so-called unstable genes. It seems that the hypothesis offered for unstable genes (DEMEREC 1935) can also be applied to 258–18. According to this hypothesis, the processes responsible for changes observed in 258–18 can be visualized as follows: As a result of X-ray treatment, a break adjacent to the roughest locus has been produced and, simultaneously with this break, changes occurred in white and roughest loci from wild type alleles to cream and roughest respectively. The chemical reactions responsible for these changes are reversible thus accounting for the appearance of cream and red, and roughest and smooth sectors. In either the unstable cream or red gene another chemical reaction may frequently be induced which is responsible for the white-cherry-red and red-cherry-cream series of changes.

All of these conditions are encountered to a greater or lesser degree in unstable gene cases which have already been described. It seems that in 258–18 the chemical changes are much more unstable than in other known cases, since in 258–18 reactions readily occur in both directions and since they are easily affected by both genetic and environmental factors.

While the change from cream into white can be explained by the loss hypothesis only if involved additional assumptions are made, such changes would be expected according to the unstable gene hypothesis. Cream to white change is comparable to alpha, beta and gamma changes described in unstable miniature of *Drosophila virilis* (DEMEREC 1929).

It is interesting to note that white, which originated by a change from cream, retained the instability of cream. This indicates that the change to "new white" belongs to the same chain of reactions which are responsible for unstable changes of cream. Experimental evidence suggests that white may be produced through several independent changes in the white locus. Now, if the new white were caused by a change in a different molecular group than the one in which unstable cream changes occur, then it would be expected that such white would cover up cream reversions, that is, that it would be stable. That was not the case in 258–18.

The origin of 258–18 (and of similar mottling characters) offers interesting possibilities for studying the interrelation between genes and genic environment. As has been mentioned earlier, in 258–18 the tip of the X

chromosome was broken to the right of the roughest locus and translocated to the inert region of the fourth. Either as a consequence of these changes or coincident with them, genes in roughest and white loci became unstable. Since it has been shown by SCHULTZ (1936) and confirmed by DUBININ (1936) and SACHAROV (1936) that a similar unstable condition is induced in certain loci when they are translocated into the proximity of the inert it appears probable that the nearness of the inert region is also responsible for the unstable conditions in the white and roughest loci of 258–18. The work of KAUFMANN and DEMEREC (1937) indicates that the chromonema of the wholly inert Y chromosome is similar in length to the chromonema of the active X chromosome. If a chromosome is visualized as a long protein molecule and bands visible in salivary gland chromosomes as markers for basic radicals forming salts with nucleic acid (WRINCH 1936), then the appearance of inert regions in salivary chromosomes suggests that these regions have relatively few basic radicals. Such regions then seem to be effective in inducing reversible reactions in certain loci.

Interdependence between changes in the genes for white and roughest loci suggest the existence of a close relationship between adjacent genes. Both this interdependence and the relation between the chromocenter and instability are additional evidence in support of position effect.

SUMMARY

258–18 is a reciprocal translocation between the X and the fourth chromosomes. The break in the X chromosomes has been located genetically to the right of the roughest locus and cytologically following band 3C4. The break in the fourth occurred in the 101F section which is close to the chromocenter.

Eyes of flies homozygous for 258–18 translocation show cream, cherry, red and roughest spotting. This spotting is interpreted as due to the instability of genes in white and roughest loci.

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CORRIGENDA

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Volume 21

Page 527, line 6, for "Chesley (1934)" read "Chesley (1935)."

Volume 22

Pages 94 to 102, for "20A" read "20B."

Page 359, line 5, for "50 percent ± aborted pollen" read "± 50 percent aborted pollen."

Page 382, figure 3, under F_2, right section, for: $\frac{(W)\ n\ r\ sp}{(Z)\ n\ R\ sp}$ read: $\frac{(W)\ n\ r\ sp}{(Z)\ n\ R\ Sp}$.

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